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CLINICAL AND EXPERIMENTAL

TUBERCLE BACILLI AND ELASTIC TISSUE FIBERS IN THE SPUTA OF DIFFERENT TYPES OF PHTHISIS*

BY ISRAEL RABINOWITZ, M.D., NEW YORK CITY

IN A RECENT publication Ellison and I have described a method for the staining of elastic tissue fibers and tubercle bacilli on the same smear. Speaking of the relationship between these two elements of the phthisical sputum we concluded that usually, the bacilli appear first, then the elastic tissue fibers, when the disease begins; also, that the elastic tissue fibers disappear before the tubercle bacilli as the process heals.

There were exceptions to this rule which I have observed in my studies of over 200 cases which we did not include in our report, because we did not consider these observations conclusive enough. I am returning to the subject now, because recent developments seem to give these exceptional observations a significance of which we had little understanding at the time we made them.

A few months ago French clinicians reported series of cases of clinical phthisis in which abacillary, respectively 'prebacillary' stages of the disease were observed. During these stages tubercle bacilli could not be demonstrated in the patients' sputa, not even by cultural or guinea pig inoculation methods.

In the material studied by myself I observed a single case in which elastic tissue fibers were present in the sputum in abundance, while tubercle bacilli could not be demonstrated not even on repeated homogenization. Cultural or guinea pig inoculation tests were not done. It was the case of a young woman who came under my observation rather shortly before her death from a Laennec type of multilobar pneumonic phthisis of about six weeks' duration. Unfortunately I could study her sputum only for a short period, I did not therefore consider the observation a conclusive one.

This case recalled to my mind another similar case which I had observed some years earlier. It was the case of a twenty-three-year-old man who was sent to the mountains with the report of an active right pulmonary process and positive sputum. He was with us hardly a few days, when he became extremely ill with a consolidation of his entire right lung. Though he had a purulent sputum, we could never demonstrate tubercle bacilli in it. At first we thought of a banal pneumonic complication, but the process soon proved to be a rapidly softening phthisical process, to which the patient succumbed in about five or six weeks. During that time we did not succeed in revealing tubercle bacilli in his sputum by the routine staining methods, no cultural or animal inoculation tests were done. I do not know whether elastic tissue fibers were expectorated in this case, as at that time we did not yet have our method for elastic tissue staining.

In the Home for Consumptives, Chestnut Hill, Pa., situated as we were in a metropolitan city, we received a large number of pneumonic phthisis patients, most of whom have been kept previously for some weeks in general hospital wards. The invariable history obtained was—that they were kept as pneumonia cases for weeks, because of negative sputa, and only just before transfer was a positive sputum obtained. These were frequently the very cases in which I found a great discrepancy between the abundance of elastic tissue fibers and the paucity of tubercle bacilli. Very frequently homogenization had to be resorted to in these cases.

Frankly speaking at the time of these observations I did not attach much significance to these findings. So much so, that in lack of precise records on these cases I could not even establish their number now. However, their number was not large enough to become conspicuous. Like other workers before me, Besancon and Brodeur,³ Hoesslin,⁴ etc., who observed such discrepancy in a few of their cases, I, too, was inclined to blame it on the accidental qualities of the specimen, faulty technique, etc., rather than assume the illogical presence of elastic tissue fibers without tubercle bacilli in the sputa of the cases in question.

Reading now the reports of the French clinicians of their cases of "abacillary phthisis," I was struck with the fact that most of their cases were also pneumonic or bronchopneumonic acute conditions. Just the type of cases in which the absence or paucity of tubercle bacilli at the earliest stages has puzzled me at the time of my studies. I came to the belated conviction that what I have observed in some of these cases was really the appearance of elastic tissue fibers preceding that of tubercle bacilli.

The question of elastic tissue fibers appearing without simultaneous presence of tubercle bacilli in phthisical sputum dates back to Sir Francis Troup, who championed the pathognomonic precedence of elastic tissue fibers over tubercle bacilli almost half a century ago. Just a few years after Koch revealed the tubercle bacillus, Sir Francis Troup published his excellent monograph on the sputum.⁵ Here, he described his natural drop method of elastic tissue examination and urged the search for elastic tissue fibers in all phthisical sputa in the following manner:

"It appears to me that the diagnostic value of early fiber is apt to be

much underestimated in the present day, when the tubercle bacillus like Aaron's rod swallows up everything else.

In many cases whose symptoms and progress called up initial phthisis into the mind, I have demonstrated elastic tissue where no tubercle bacilli were found, and in one remarkable case I had the opportunity of watching and seeing the advent of the tubercle bacilli months after elastic tissue was first noticed. Since the author became acquainted with Koch's discovery he has carefully examined many hundreds of sputa and has arrived at the conclusion that curly fiber is actually a prodrome a precursor of the bacillus.

In ancient, long spun out cases of phthisis, tissue is also to be found pretty constantly though sparingly and in minute morsels, while the bacilli may be missed entirely or for long intervals. Then he goes on relating cases observed by him in which elastic tissue preceded the appearance of the bacilli and it is quite apparent that the cases were mostly acute pneumonic conditions similar to those I have above mentioned.

By all appearances recent developments of phthisiology point to the fact that the contentions of Sir Francis Truup are about to be vindicated. It took nearly half a century to come to the appreciation of his unheeded observations. While it is not yet possible to say that his contentions have been proved, evidence is coming forth tending to show that there is a type of phthisis in which elastic tissue fibers are expectorated without tubercle bacilli.

The conception of the French clinicians is that in some phthisical conditions the bacillus passes through a filterable invisible, ultravirus form before it takes up its usual acid fast form. This transformation of the tubercle bacillus accounts then for its absence in the so called pre or abacillary stages of the disease.

Whether or not we accept the assumption of the French clinicians as regards the ultravirus of the Koch bacillus, our particular question here turns on the point of whether or not in these conditions elastic tissue fibers appear in the sputum before the tubercle bacilli. Information as regards this question is meager, for the simple reason that these conditions are mostly observed in the general wards where investigation of the sputa for elastic tissue fibers is not usually done.

Of similarly great interest is the other contention of Sir Francis Truup, that in chronic cases the disappearance of elastic tissue fibers follows that of the bacilli, and that "minute morsels" of elastic tissue may still be demonstrated when no more bacilli are to be found. In our quoted publication Elhson and I have expressed ourselves in agreement with former workers, stating that as a rule the elastic tissue fibers disappear before the tubercle bacilli. However, in that publication we restricted our report to the two main and most frequent types of elastic tissue elements. We did mention that Besancon and Brodriez described other forms besides, which we have left unconsidered for the sake of simplicity. Besancon and Brodriez described an amorphous form of elastic tissue elements which comes in smaller or larger patches. In the study of my own material I have also observed amorphous forms of elastic tissue elements in single morsels or heaped patches, which I found to be definitely pathognomonic of old emphysematous phthisis. I found this

form to appear quite frequently even in already long arrested negative sputum cases, in which only Much's granules could be demonstrated but never whole bacilli. Assuming as I do that these are the elements Trioup is referring to with his "minute moissels," I think that my observations are bearing out the contention of Trioup to the full. Besancon and Biodiez, who emphasize that in their experience elastic tissue always disappeared before the bacilli, also mention the persistence of the amorphous elastic tissue elements after the bacilli have already disappeared, in some of their cases.

Summing up, I must emphasize here—that I fully realize the inconclusiveness of my studies as far as their bearing on the here-discussed questions are concerned. The purpose of this communication is rather to call attention to these most interesting questions, and stimulate investigation in this direction. It seems to me that such investigations should be carried out in the general hospitals, where these atypical pneumonic processes are usually found in the earliest stages. While the relations of disappearance of the elastic tissues and the tubercle bacilli should be studied in the tuberculosis wards, all the more so—because they have, in my opinion, the greatest bearing on prognosis in phthisis.

Another question of great interest is the clinical significance of the relative quantities of elastic tissue fibers as compared with the quantities of tubercle bacilli. It is quite evident that only extremes in the positive as well as negative direction are of any significance. Since elastic tissue expectoration means pulmonary tissue destruction it seemed very logical to me that its persistence, even if not excessive, should speak for progress of the disease rather than persisting bacillus expectoration, which may only signify an open lesion without spread. Careful investigations of this question with my material, with serial x-ray control and comparison of parallel cases, led me to the following preliminary conclusions. Excessive amount of elastic tissue expectoration invariably means rapid softening and caseation of extensive lesions, or cavitation of confluent proliferative lesions of a more chronic character.

My first thought was to identify excessive elastic tissue expectoration with extensive, now designated as exudative, caseous processes. My observations have not borne out this assumption. Widespread genuinely proliferative lesions lead to vast amount of elastic tissue expectoration just as frequently as do exudative lesions, and may be even more so. I have observed this in some chronic proliferative cases over considerable periods, frequently just before extensive fibrosis began and the process took a favorable turn. The most remarkable thing about these cases was—that the patients had no clinical signs of activity or such signs were just abating, while elastic tissue fibers continued to stream out in great abundance. On the other hand I have observed cases of the exudative type in which a sudden excessive expectoration of elastic tissue fibers coinciding with clinically very active periods, enabled me to foretell coming on hemorrhages. In fact, I lost two patients in fulminant pulmonary hemorrhages just after the appearance of such excessive elastic tissue expectoration. It would seem therefore that the amount of elastic

tissue expectorated must also be judged in the light of other clinical phenomena in each individual case

I believe, however, that persistent elastic tissue expectoration, in cases with or without mild symptoms of clinical activity, has greater significance than the mere expectoration of bacilli has. I found that such cases progressed to slow cavitation, the course of which was chronic but definitely destructive in the long run. The longer such elastic tissue expectoration lasted the lesser were the chances of the process going to spontaneous healing. On the other hand, in cases showing chronic low grade activity or even moderate degrees of activity in which the sputa showed gradual disappearance of elastic tissue fibers, even if bacillus expectoration continued undiminished the x rays soon showed definite signs of absorption and with more or less delay these cases came to clinical arrest. In one word periodical check up of the elastic tissue expectoration permitted far reaching prognostic predications which were borne out by the events to a remarkable degree.

After a while, these studies of the elastic tissue behavior in the different stages of the various conditions enabled us to compare types and stages of phthisical conditions from the standpoint of elastic tissue findings. Frequently we were able to make even a diagnosis of the particular phase or type of the disease from the sputum examination alone, as supported by the clinical history of the case in question.

It is my firm conviction that large scale investigation with a standard of counting and comparing expectorated quantities of elastic tissue elements as observed and checked up periodically, would reveal such regularities typical to certain types and phases of phthisis as to permit definite diagnosis and prognosis in a large number of cases. It would also enable us to set up such type and phase classifications of phthisis that would be of more value from a practical point of view, than the usually accepted divisions of today.

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121 WEST EIGHTY EIGHTH STREET

INCIDENCE OF BACTERIA IN SPUTUM CULTURES FROM FIFTY PATIENTS HAVING ASTHMATIC BRONCHITIS*

A PRELIMINARY REPORT

BY MARGARET J. MCKINNEY, M.S., CHICAGO, ILL.

I

IN THE preparation of sputum vaccines used in the treatment of certain asthma patients, there occurs the opportunity of studying the type of bacteria resulting from sputum cultures. The sputums studied were obtained from 50 patients having asthma, who were not sensitive to proteins used in routine skin testing, and whose history showed the original asthmatic attack to have followed bronchitis or a "cold."

II

Koessler and Moody¹ in 1917 made vaccines from both aerobic and anaerobic cultures of sputum from patients having bronchial asthma, and Huber and Koessler² in 1922 mention such vaccines in treatment of a case of bronchial asthma. The organisms obtained by them from aerobic cultures were pneumococci, streptococci, the influenza bacillus, and *M. catarrhalis*. Rackemann³ in 1920 reported the growth, from sputum cultures from asthma patients having bacterial infections of the bronchi, of nonhemolytic and hemolytic streptococci, *Staphylococcus albus* and *aureus*, and gram negative bacillus and a gram negative coccus. Rackemann and Graham⁴ in 1924 reported further work on vaccines from asthma sputums, with growth of organisms occurring in the following importance, green streptococci, nonhemolytic streptococci, staphylococci, pneumococci, *B. influenzae*, and a gram-negative bacillus. Rackemann and Seully⁵ in 1928 found again that green and hemolytic streptococci, and staphylococci seemed to predominate in asthma sputum, with growth also of pneumococci and *B. influenzae*. Thomas, Famulener and Touart⁶ in 1924 identified organisms from asthma sputum cultures, finding a predominance of streptococci, green, hemolytic and indefinite, in the order named, with staphylococci second in number. Other organisms recovered were atypical gram-negative cocci and bacilli, pneumococci, *M. catarrhalis*, enterococcus, *B. coli communis*, *B. fecalis alcaligenes*, and Friedlander's bacillus. Wherry⁷ in 1927 identified the organisms from asthma as *M. aureus*, *M. albus*, *M. flavus*, *M. catarrhalis*, *Streptococcus anhemolyticus*, *Pneumococcus I, II, III, and IV*, *B. influenzae*, *B. mucosus* (Friedlander's), *B. duplex liquefaciens*, and *B. abortus*. Walker and Adkinson⁸ (1928), in a report covering several years of work on sputum from asthmatic bronchitis record the predominance of streptococci differentiating the strains, with staphylococci following in importance, other bacteria not being classified.

*From the Asthma Clinic of Northwestern University Medical School.
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Noble, Fisher and Brameid in a recent article on acute respiratory infections, have made a study of aerobic flora from both normal and infected respiratory tracts. They find that the normal respiratory flora varies with the subject and the time of culture. However the average basic flora consists of green streptococci and gram negative cocci. Frequent transient organisms are streptococci, *B. influenzae* staphylococci and diphtheroids. Occasional transients are hemolytic streptococci, *M. catarrhalis* and Friedlander's bacillus.

III

The technique of making the sputum cultures was as follows. Sputum raised by the patient during an asthmatic attack was collected in a sterile container and taken as soon as possible to the laboratory. Using a looped inoculating needle, portions of the sputum were washed by gently shaking the inoculated loop in sterile physiologic salt solution. The washed loopful of sputum were then streaked in subburst pattern on blood agar plates (5 per cent defibrinated human blood) and were also inoculated into tubes of plain and asetic fluid broth. After incubation for twenty four hours at 37° C. the cultures were examined. Colonies were picked from the plates for subculture on other blood agar plates, broth cultures were streaked on blood agar plates and these cultures were examined and subcultured after suitable incubation. Pure cultures were transferred to various sugar media for identification of the organisms. Vaccines were made by washing pure cultures on plain agar slants or in plain broth with sterile physiologic salt solution, counting by comparison with a known number of red blood cells, diluting to the desired number of organisms per cubic centimeter and heating in the water bath at 56° C. until all organisms were killed.

Streptococci were cultured in broth after their character on blood agar was determined. They have been classed here only as hemolytic and non hemolytic, the green producing strains being included in the nonhemolytic group. Further identification of streptococci is reserved for a later discussion.

Many gram positive diplococci were recovered and were tested for bile solubility. No organisms having all the characters of the pneumococci were cultured.

Organisms of the staphylococcus, neisseria, *Mucosus capsulatus* and diphtheroid groups grew well from initial cultures on blood agar. Other organisms mentioned were obtained also from initial plate culture. No attempt was made to isolate the influenza bacillus by means of special media.

IV

Table I shows the type and number of organisms recovered from sputum cultures. Streptococci chiefly nonhemolytic predominated in culture. The neisseria group, gram positive diplococci (bile insoluble), and staphylococci followed in the order named. Other organisms recovered were the *Mucosus capsulatus* group (including *Bact. pneumoniae*), diphtheroids, gram positive cocci (as *M. tetragenous*), yeast like organisms, *Bacillus subtilis*, streptothrix, and a vibrio.

TABLE I
INCIDENCE OF BACTERIA IN FIFTY ASTHMATIC SPUTUMS

ORGANISMS CULTURED		NUMBER	TOTAL NUMBER	PER CENT
Streptococci	Nonhemolytic	38	43	23.62
	Hemolytic	5		
Neisseria	N. crassus	2	32	17.58
	N. sicca	2		
	N. catarrhalis	1		
	Others	27		
Gram positive diplococci (bile insol.)			27	14.83
Staphylococci	Staph. albus	19	23	12.63
	Staph. aureus	4		
Mucosus capsulatus group	Bact. pneumoniae	4	14	7.69
	Others	10		
Diphtheroids	C. Hoagii	2	14	7.69
	C. xerosis	1		
	C. flavidus	1		
	C. Hoffmanni	1		
	Others	9		
Gram + cocci	M. tetragenous	2	8	4.39
	Others	6		
Yeast like organisms			9	4.94
Bacillus subtilis			8	4.39
Streptothrix			3	1.64
Vibrio group			1	0.54
Total number of organisms cultured			182	99.94

V

The results of this study are in general accord with previous investigations on asthma sputums in finding a predominance of streptococci in culture. These results are slightly different, however, in placing the gram-negative cocci (neisseria group) second in number, and the staphylococci fourth. How many of the organisms recovered belonged to the normal mouth flora of the patients was not determined.

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BASAL UREA ELIMINATION

By DR CHARLES RICHT, Dr * PARIS, FRANCE

IN A DOZEN contributions published during the last three years I have presented my studies of a new conception which in French I have termed *excrétion basale*¹. In English the expression basal urea elimination seems to express the same idea. This concept in its own field is analogous to that of basal metabolism as presented by du Bois.

It is well known that the basal metabolism represents the heat produced per square meter body surface per hour with the individual at rest at an optimal temperature and fasting for at least twelve hours.

I have formulated a similar definition of basal urea elimination. *Basal urea elimination is measured in terms of the elimination of urea per square meter body surface over a period of twenty four hours with the individual in an ideal state of nitrogen equilibrium.*

Body surface is measured by du Bois' formula for men and women or by Rubner's formula for dogs. We have measured the urea elimination rather than the nitrogen elimination for many reasons. The former determination is much simpler in medical practice. The relation between the urea nitrogen and the total nitrogen elimination in urine is always constant†. With subjects in good health and in nitrogen starvation we neglect the 5 per cent nitrogen elimination in the stools.

The chief consideration in the determination of basal urea elimination is dietary. In our experiments dogs have been put successively on the following diets: starvation, sugar diet, fat diet, and sugar and fat diet. From these studies we found that urea elimination is minimal on a combined fat and sugar diet (six grams of sugar and six grams of fat per kilogram body weight). Dogs which could not tolerate this diet without diarrhea were eliminated. As a rule the urea elimination became stabilized by the ninth or tenth day, after which it remained constant. We found that with dogs on this diet the urea elimination per kilogram was variable, but when we expressed it in relationship to square meters of body surface we found that the elimination became regular.

The urea elimination per square meter is always about the same (about 7.65 grams).

In our bibliographic research we found that Irwin Voit's results were comparable. This author found that three dogs of 29, 18 and 7 kilograms weight respectively, showed a nitrogen equilibrium during starvation of 5.2, 6.6 and 5.2 grams per square meter per twenty four hours.

Professor Agrégé de Physiologie à la Faculté de Médecine de Paris

Received for publication February 1 1929

†It is lower in nitrogen starvation than on the usual diet. I found it reduced on the average 70% in man and 75% in the dogs.

TABLE I

	ELIMINATION OF UREA IN GRAMS PER SQUARE METER PER 24 HOURS	ELIMINATION OF UREA IN GRAMS PER KILO PER 24 HOURS
Dogs weighing more than ten kilo grams (4 dogs)	7.6	0.33
Dogs weighing nine kilograms (4 dogs)	8.4	0.44
Dogs weighing from seven to nine kilograms (10 dogs)	7.4	0.41
Dogs weighing less than seven kilograms (6 dogs)	7.5	0.46

In view of these findings I have formulated the following law *In nitrogen equilibrium the urea elimination is proportional to the surface and not to body weight*

But for man it was impossible to give such an alimentation quite free from protein, limited entirely to sugar, starch and fat. We lost hope of applying these experiments to man when unexpectedly we made the following verification.

If a man has in his dietary a sufficient number of calories (about three thousand per day), nitrogen equilibrium is the same whether his intake consists of eight, ten or fourteen grams of protein. Nitrogen elimination in any of these cases corresponds to the catabolism of nineteen grams of protein (wear and tear quota of Rubner). If it is impossible to feed a man for ten or twelve days without protein, it is not difficult to provide him with a diet which he can tolerate and which contains only twelve to fourteen grams of protein*. This we did on ten adults and twelve children whom we put on such a diet, it enabled us to obtain satisfactory results.

The following standard dietary was given: sugar, starch, all kinds of fruit, nuts (except chestnuts, walnuts, hazelnuts and almonds), jam, potatoes in moderation as they contain 2 per cent protein, carrots, butter, oil, Devonshire cream.

The major portion of the starch is contained in a special bread. Starch (100 grams) is mixed with butter (60 grams) and sugar (90 grams). Just enough water is added to dissolve the sugar. The mixture is raised with a little yeast for twenty-four hours to make it light. It is baked in a hot oven. This bread is highly nutritious, and one has no difficulty in eating 200 grams a day. Its caloric value is about 500 calories per 100 grams.

On this diet, nitrogen equilibrium is established by from the seventh to the tenth day. For the next three days the urea elimination is measured. With this method we have found that the elimination of urea per square meter per day for an adult averages 2.5 grams.

Individual differences may be of importance. Figures for the normal may range between 1.9 grams and 3.1 grams or roughly between 2 and 3 grams per square meter per twenty-four hours. For instance the first three persons studied were three adult doctors in good health and of great physical activity.

*With the food tables appended at the end of Lusk's book "The Science of Nutrition" it is easy to arrange a diet containing more than 2500 calories and less than 14 grams of protein.

Their food contained on the average 13.4 grams, 13.9 grams, 14.1 grams of protein per day, and the basal urea elimination was respectively 2.8 grams, 2.53 grams and 2.04 grams. We must admit then that the number 2.5 grams per square meter per day may vary as much as 20 per cent in either direction. The individual differences in this determination appear to be greater than the normal variation in basal metabolism determination, where they do not exceed 10 per cent.

Individual variations are greater than class differences, such as the variation between men and women (2.41 and 2.91), between people past the age of forty five years and persons from ages twenty eight to forty five years (2.39 and 2.74), between workers and those who are at rest in bed (2.46 and 2.56), between those who eat ten grams or less of protein per twenty four hours and

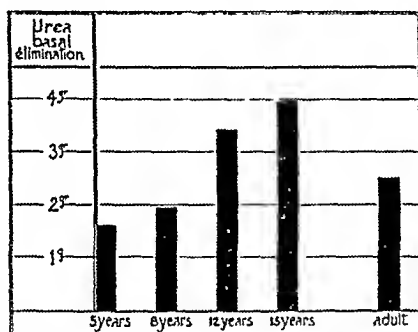


Chart I—This schema shows the variations of urea basal elimination at different ages. We see that it increases from five to fifteen years of age and that afterward it decreases.

those who eat between ten and fourteen grams (2.39 and 2.81), and between those who weigh either more or less than sixty kilograms (2.56 and 2.46).

For children the basal urea elimination is not the same as for adults, as is shown in Table II.

TABLE II

AGE OF THE CHILDREN	BASAL UREA ELIMINATION
From 4 to 6 years (4 children)	1.6
From 7 to 9 years (4 children)	1.97
From 10 to 12 years (2 children)	3.46
From 13 to 14 years (2 children)	3.9

I conclude from these figures that children have a basal urea elimination which varies with increasing age. Slight in the first years, it increases suddenly at about the tenth year and seems to be maximal toward the fifteenth year. The accompanying graphic chart shows this phenomenon well.

What is the biologic value of basal urea elimination? The basal urea elimination represents the elimination of urea per square meter area with a protein diet insufficient to cover the needs of the organism, but containing a

sufficient number of calories for maintenance. On such a diet instead of having an excess consumption of protein, one has a dearth consumption. As a consequence the organism will burn a portion of its own protein in order to provide maintenance of life. Since the caloric needs are covered, the body will burn just the quantity necessary for maintenance.

The measurement of this nitrogen elimination records quantitatively the wear and tear or better the cellular needs of the organism.

CONCLUSION

The basal urea elimination is the quantity of urea eliminated per day per square meter body surface in subjects on a diet providing ample caloric value but insufficient in protein.

This amounts in dogs to 7.65 grams, in human adults 2.50 grams, varying between 2 and 3 grams. In children it is about 1.65 grams at five years of age, 2 grams at eight years, 3.4 grams at eleven years, and 4 grams at fourteen years, with important individual variations.

The study of the basal urea elimination seems to us to offer promise of distinct value in studies in physiology, in which domain I have been carrying on my investigations, and in pathology, where it is still, I think, a no man's land.

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THE URIC ACID INCREASE IN THE BLOOD OF PATIENTS WITH CARDIAC DECOMPENSATION

By I. LISA WILLIAMS M.D. CHICAGO III

THE uric acid content of the blood is increased in many diseases. Most of the studies have been made on such diseases as gout, nephritis, hypertension, arteriosclerosis, strabismus, epilepsy or acute intoxications in which the kidneys have been damaged and the increased blood uric acid could be explained on the basis of deficient elimination.

In a previous communication¹ it was pointed out that in the blood of patients with chronic nephritis and hypertension and with myocardial insufficiency there was a much higher concentration of uric acid than in those whose cardiac function was adequate. It was also stated that with clinical improvement there was a diminution in the concentration of uric acid in the blood. Other than this report no systematic study of the blood uric acid of patients with heart disease but without nephritis is to be found in the literature.

This study was made on patients in varying stages of cardiac decompensation but without nephritis as determined by careful clinical study, functional tests, frequent and repeated examinations of the urine and in several instances by postmortem examinations. During the past six years 22 patients were selected and studied. There are 14 males and 8 females in the group. The ages varied from eight to seventy years and the average was about forty years.

The clinical diagnosis was chronic myocarditis with 8 patients, 6 of whom had hypertension. Thirteen had chronic valvular disease of the heart. Of these, 8 had mitral stenosis either alone or combined with mitral regurgitation. In 4 patients there was aortic regurgitation and in one both aortic stenosis and regurgitation. Mitral regurgitation was the sole lesion detected in one patient. In another syphilitic aortitis with mitral regurgitation was the diagnosis.

The blood uric acid was determined in all patients and with several, repeated determinations (five in one instance) were made. The blood urea nitrogen, nonprotein nitrogen and creatinine were also estimated in almost every case. The carbon dioxide combining power of the plasma was measured in several patients and the amino acid nitrogen of the blood of a few was also recorded.

The blood uric acid varied from 3.0 to 9.8 mg. in the maximum range of all patients and all determinations but the averages for these patients ranged from 3.6 to 6.9 mg. with a grand average of 4.75 mg. per 100 cc. of blood.

The averages for blood urea nitrogen were 9.4 to 45.7 mg. with a final average of 20.8 mg. per 100 cc. For nonprotein nitrogen these values were 27.4 to 66.7 and 40.1 mg. as a grand average. For creatinine the range was

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11 to 21 mg with 15 mg for the mean. The figures for plasma bicarbonate (6 patients) were 53.9 to 73.6 and an average of 59.2 vol per cent. The amino acid nitrogen was determined in 15 patients and the mean values ranged from 6.0 to 8.7 mg with 7.4 mg as the average.

The average maximum and minimum and the grand averages are summarized in Table I.

TABLE I

AVERAGES*	URIC ACID	URIC NITROGEN	TOTAL N P N	CREATININE	AMINO ACID NITROGEN	CO COMB POWER
Maximum	6.9	45.7	16.7	2.1	8.7	74.6
Minimum	3.6	9.4	27.4	1.1	6.0	53.9
Mean	4.75	20.8	40.1	1.5	7.4	59.2

*Uric acid, urea, nitrogen, total nonprotein nitrogen, creatinine and amino acid nitrogen in mg per 100 cc. CO combining power in vol per cent.

For the determination of the various nitrogenous constituents the methods of Folin and Wu,² and of Folin³ were used except that for blood urea nitrogen Van Slyke and Cullen's⁴ modification of Marshall's urease method was used. For ure acid the silver lactate precipitation method and Folin's⁵ later modification were used. The direct method was found to yield results 0.2 to 0.5 mg higher than those reported. For the estimation of the alkali reserve Van Slyke's method was used.

The systolic blood pressure varied from 88 to 240 mm, the average of the maximum blood pressure recorded being 157 mm of mercury, the average of the minimum being 138 mm. The diastolic pressure varied from 50 to 140 mm of mercury.

The phenolsulphonaphthalein test was done on several patients and varied from 50 to 65 per cent for two hours.

The urine was examined repeatedly both during the stage of decompensation and also during improvement. Albumin was present in all but three at some time during the period of decompensation but was absent in all for at least one test during the periods of recovery or improved compensation. Likewise in many patients casts or red blood cells were detected microscopically in the urine sediment during the time when cardiac decompensation was evident.

The Wassermann test was made on the blood of all the patients and was positive in four, all of whom gave other clinical evidence of syphilis.

Dyspnea was the one symptom present in all the patients and was the chief symptom in 19 patients. Orthopnea was marked in 7 patients. Cough was prominent in 14 patients. Six patients complained of palpitation of the heart and in 5 precordial pain or distress was mentioned. Dizziness was present in 4 patients and weakness was pronounced in 5.

Enlargement of the heart was the most important physical abnormality noted and was present in all but one, the enlargement however being only slight with 4 patients. Edema of the lower extremities and trunk was quite marked in 13 patients and was present at times in several others.

Enlargement of the liver was demonstrable in 20 patients. In the 2 patients in whom the liver was not palpable, the blood ure acid was the lowest of the entire series, the values being 3.3 and 3.7 mg per 100 cc.

Signs of pulmonary hypostasis or hydrothorax were definite in 14 patients at some time during the clinical course studied.

Of the 22 patients 10 were known to be dead after a period from one month to four years. Only 4 were known to be improved, 3 were in worse condition when last heard from, 3 were unchanged and 2 could not be followed after a few months.

Of the 10 patients known to be dead all had clinical evidence of a failing heart as the terminal picture. One had an associated pneumonia. Postmortem examination of 5 disclosed as the cause of death, heart disease with associated passive hyperemia of the lungs, liver and kidneys. Microscopic examination of the kidneys revealed changes associated with passive congestion but no appreciable nephritis was found.

Nine patients were studied during the periods of marked cardiac decompensation and also during remissions when definite improvement was noted. The averages are grouped in Tables II and III.

TABLE II
PATIENTS WITH MARKED DECOMPENSATION

MG PEP 100 CC	UREA N	N P N	URIC ACID	CREATININE	AMINO ACID N
1	23.6	43.7	—	1.1	
2	19.6	33.8	4.1	—	—
3	38.4	9.9	7.3	—	—
4	28.4	10	—	—	—
5	75	94	9.4	2.3	
7	21.0	41	4.8	1.9	
8	18.3	38.3	6.3	1.6	7.7
10	21.8	44.7	4.4	1.4	8
22	19.5	36.0	4.1	1.7	6.7
Average	29.6	49.0	9	1.7	7.7

TABLE III
PATIENTS DURING IMPROVEMENT

MG PEP 100 CC	UREA N	N P N	URIC ACID	CREATININE	AMINO ACID N
1	22.5	38.9	4.6	1.8	
2	16.2	34.1	3.6	2	
3	28.6	47.1	6.4	1.9	
4	13.8	31.3	3.1		
5	15.8	34.7	3.3		
7	17.1	39.4	3.6	1.6	
8	19.8	41.4	5.2	1.4	
10	22.9	44.4	4.2	1.2	7.5
22	23.3	37.3	3.0	1.6	6.6
Average	19.9	38.5	4.1	1.7	7.1

From these averages it is apparent that during improvement there is a decreased concentration of all the nitrogenous waste products of the blood except creatinine. The average decrease in blood urea nitrogen was 9.7 mg of nonprotein nitrogen it was 10.5 mg and of uric acid it was 1.8 mg per 100 cc but whereas the relative percentage decrease in nonprotein nitrogen was 22 per cent and of urea nitrogen 32 per cent that of the uric acid was 44 per cent and thus in spite of the well known fact that uric acid is the most difficult of

all these substances to be excreted by the kidneys. That other factors than a mere improvement in the circulation of the kidneys, are involved, seems likely.

With the evident improvement of the cardiac decompensation in these 9 patients there was also a marked diminution in edema and in the size of the liver. With some patients the liver margin receded several centimeters as compensation was regained and concomitant with this change there was a considerable decrease in the amount of circulating uric acid, also when heart failure again supervened and death approached, the opposite condition again prevailed. The amino acid nitrogen of the blood was studied in 3 patients and in two of these it was decreased in its concentration.

In diseases of the liver in which there is a considerable destruction of liver tissue, such as eclampsia, acute yellow atrophy and hyperemesis gravidarum, there is a marked increase in the concentration of uric acid in the blood. That the liver has something to do with uric acid metabolism is accepted by most workers in this field. Folin and associates⁶ found in the dog that an Eck fistula is without effect upon the destruction of uric acid intravenously injected. However in liver extirpation experiments as recorded by Perroneito,⁷ and Bollmann, Mann, and Magath,⁸ the level of circulating uric acid rises rapidly when this organ has been removed in spite of the presence of intact kidneys. Thus it might appear reasonable to ascribe some of the retained uric acid in the blood of patients with marked cardiac insufficiency to the impairment of the function of a liver, partially incapacitated by extreme venous engorgement.

The diagnostic value of the quantitative analysis of the blood for various nonprotein nitrogen extractions is well recognized in nephritis. That relatively high levels of these substances may be present in the circulating blood of patients without nephritis is evident from a study of Table II, where No. 5 has a nonprotein nitrogen of 94.5 mg per 100 cc. The absence of nephritis in this patient was verified by postmortem examination. Careful clinical study and repeated chemical analyses of the blood had previously demonstrated normal functioning kidneys when cardiac compensation was restored. On one such occasion the blood uric acid was 2.2 mg per 100 cc. The other nitrogenous waste products were normal in amount and the urine was free from albumin and casts.

In 16 of the 22 patients normal values for the various nitrogenous substances other than acid were obtained on at least one occasion. In 5 of the 6 others the values were only slightly elevated and with one a moderate increase was demonstrated at some one period of study.

CONCLUSIONS

- 1 Cardiac decompensation without nephritis is associated with a marked increase in the concentration of blood uric acid and a moderate increase in urea and nonprotein nitrogen.

- 2 There is a marked decrease in the concentration of uric acid and a moderate decrease in urea and nonprotein nitrogen of the blood following improvement in cardiac efficiency.

- 3 This decrease is accompanied by a diminution in the size of the liver and by an improvement in edema.

4. The results cited here support the hypothesis that the liver is an important organ in nitro acid metabolism.

5. The determination of the blood urea acid together with the other non-protein nitrogen extractives of the blood is of diagnostic value only when combined with careful clinical study and repeated analysis.

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EXPERIMENTAL ILLUMINATION OF ADHESIONS CAUSED BY INTRAPERITONEAL INDUCTION OF NEOARSPHENAMINE*

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THE present report is based on the autopsy results from 67 animals injected intraperitoneally two to six times with solutions containing one per cent neoarsphenamine.

LITERATURE

Favorable clinical results in infants are reported in 25 out of 28 cases^{1, 2, 3, 4}. The incidence of adhesion formation in infants, however, is not yet established since there are only three reported autopsies^{4, 5}. One infant showed no adhesions following six injections, one was free from adhesions following only 2 injections, and the third died from peritonitis following an accidental injection into the rectum sheath. A clinical search for adhesions was reported in 2 cases³ but the negative results have little weight since surgeons agree⁶ that abdominal adhesions rarely cause symptoms although they may eventually produce chronic pain or even acute intestinal obstruction and since only those adhesions which distort the stomach or colon are commonly diagnosed by the x-ray.

The animal experiments reported to date include only results in rabbits and the early, incomplete results of the present series.⁴ Rosenberg's results⁴ from 35 rabbits include a temporary chemical peritonitis, pericapsulitis and perisplenitis, some manifestations of discomfort for a few hours and "in rare instances" adhesion formation. Yampolsky and Kling⁷ reported that rabbits receiving 10 injections of neoarsphenamine were "still alive and healthy," while "some of those" receiving alternate injections of neoarsphenamine and mercurous iodine died and showed no adhesions.

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A knowledge of the present conception of the physiology of absorption from the peritoneal cavity of the pathogenesis and prevention of adhesions, and of the pharmacology of neoarsphenamine was, of course, fundamental to the present study. Cunningham,⁴ Hertzler,⁵ Voegtlin,⁶ and others,⁷⁻¹⁰ have recently written excellent critical reviews of the literature in these fields.

EXPERIMENTS

Our initial quest was finding the species most subject to the formation of adhesions after intraperitoneal injections of neoarsphenamine. The procedure used throughout these experiments included taking full sterile precautions in preparing solutions and in making injections, basing dosage on 0.012 gm. of neoarsphenamine per kilogram body weight in all animals (first injection was always three fourths of this dose) using in practically all cases a one per cent strength and a seven day interval, securing numerous controls by using the contents of a single ampoule of neoarsphenamine in animals from several different experiments, noting carefully the animal's reactions, health, and stools, and performing complete autopsies after a varying number of injections and at varying time intervals after the last injection. The temporary inflammatory changes found were similar but less severe than those of Rosenberg¹ and are ignored in this report because they were influenced by the variation in time between the last injection and the autopsy. Since single injections did not produce permanent changes, they are also omitted. Variations in the degree of histologic changes in the extensive and varied peritoneal surfaces were found very difficult to compare accordingly the experiments were gradually judged more and more on the basis of adhesion formation. This seemed to be the only permanent macroscopic change. The causes of death other than experimental or pneumonia (incidence about the same as in animals used in other experiments) are also mentioned.

Eight guinea pigs, 10 rabbits, and 6 white rats, injected 3 to 6 times each, showed no formation of adhesions. The only pathology found was thickening and engorgement of the peritoneum about the site of injection in one rabbit. The "rare instances" of formation of adhesions in rabbits reported by Rosenberg¹ may have been due to a larger series, to a stronger (2 per cent) solution or, possibly, to a more irritant lot of neoarsphenamine.

In sharp contrast to the above results, 6 out of 7 dogs showed the formation of adhesions. Four of these dogs had definite but harmless adhesions following 2 to 6 injections when autopsied fourteen to thirty seven days after the first injection. Dog 31 was kept seventy five days and had no adhesions. The more interesting changes in the other two included:

Dog 27, four injections, twenty three days (between first injection and death), four adhesions of the omentum to itself formed masses 1 by 1 by 0.5, 3.5 by 1 by 1, 5 by 0.5 by 0.5, and 2.5 by 1 by 0.5 cm., respectively, all containing some fibrous tissue, the largest being engorged with blood, four adhesions between the intestinal serosa, the mesentery, and the omentum, and the rhum was caught in a short loop by a firm adhesion in such a way that an obstruction might easily have occurred,

Dog 30, six injections, thirty one days, extensive adhesions matted all the peritoneal organs together, a condition which was gradually starving the

due to death, this was probably due to the rupture of the transient mesodermic adhesions when there was a paralytic flow caused by an ischaemic attempt to withdraw spinal fluid from the fourth ventricle, both radicle cuticles were inactive.

After the completion of the other 4 post-mortem tests reported, an opportunity arose for the 5th experiment, on cat. Adhesions were seen at 14, 15 and 16 of the 7 cats included, though in only two were the adhesions at all marked, and in none were they regarded as to impair health. That 14 had 10 or 11, partly, slightly, chiefly fixed in the post-mortem cavity and periphery of mesodermic adhesions at the equator to the sphere. The 5 findings coincide with Hall's view that only temporary adhesions are formed when an embryo adheres to an attached cell and causes a large expansion, while permanent adhesions result from the destruction of an attached cell in the absence of an inflammatory reaction. That most of the adhesions found were of the temporary type is further supported by that 14 where the cleavage of two days after the last injection may have been a basic factor in the absence of adhesion. Temporary adhesions, however, may become permanent by the growth of fibroblasts into the blast.

The death of that 16 from secondary septicæ caused by continued moist saline extraperitoneal hypertension 10 by 14 by 15 on, was probably due to an accidental infection of the mesodermic into the abdominal wall. This confirms the danger from highly tuberculous chorio by the death of an infant from a similar cause. A small, hard, sub-acute, brownish, found in 14 and 16 was the only indication in the entire series that the sharp middle point had directly injured the intestine. It is interesting to note that the same operator had already made some 20 successful intraperitoneal injections into animals by two these two incidents occurred. Cats, however, were much more difficult to hold during the injection and would often require sedation when they were apparently tightly and comfortably held.

The sharp contrast between the formation of adhesions in cats and rats and the complete absence of adhesions in the other laboratory animals is probably due to difference in the conditions which was involved in these results of the latter two. The kitten put but very little resistance. In the rabbit the body of the omentum is retroflected and it cannot reach into the lower third of the abdomen. The liver also, however, is very active in adhesive formation as has been shown by Nodder and Winer.¹¹ In white rats the omentum is extensive but very delicate. Rubin in his careful studies on the omentum states that this organ as well as its part in the formation of adhesions is very similar in rats, dogs, and man. The absence of adhesions in fishes¹² may be due partly to difference in size since size influences the rate of absorption and the reaction to infection.¹³ A comparison of paper and inkish show would be instructive.

Our next attempt was to stimulate the formation of adhesions. Among the many methods suggested by various authors¹⁴⁻¹⁷ the method thought to be the most applicable to the present problem was the postural separation of surface plates to adhere. Because of the frequent involvement of the omentum and the lack of spontaneous mobility,¹⁸ it was thought that keeping the injected solution away from the usual location of this organ might reduce

the incidence of adhesion formation. For this purpose, dogs were kept on their backs in a "Fowler's position" (20° incline) for twelve hours after each injection into the lower left quadrant.

Adhesions were found in 4 of the 6 dogs injected. These adhesions, however, were not as marked as those obtained when the dogs were unrestrained. These were the only animals in the entire series showing any distress after injection. Their vomiting, however, was due to the injection being given in the evening when their stomachs were full, and was easily corrected. In three dogs the omentum was stretched clear to the symphysis and held by firm adhesions. This unusual condition is probably explained by the posture and by the use of morphine (for quieting), tending to favor a displacement of the viscera toward the symphysis.

Although keeping the solution away from the common site of the omentum materially reduced the incidence and degree of formation of adhesions, this method has little clinical value since low insertions of the omentum frequently cause chronic distress by pulling on the transverse colon and stomach.¹⁴

We next attempted to diminish adhesion formation by varying the solution injected. Variations in the tonicity of the solution and in the concentration of neoarsphenamine were tried because of their influence on the rate of absorption, on the exudation of body colloids, and possibly, on the direct damage to the mesothelial cells.⁶ The hydrogen ion concentration was varied because of its effect on the rate of oxidation and the coincident toxicity of arsphenamines.⁸ Several solutions were used as vehicles because they might either reduce the irritant properties of neoarsphenamine or interfere with the formation of adhesions. As many experiments of each type as could be included in a single series were run to get an indication as to which theory should be tested more fully. When the striking results from the use of sodium bicarbonate were noted, all other experiments were stopped to run a series of such animals. As the problem had to be dropped shortly after this, the incomplete results from the other solutions are valuable chiefly as controls.

All solutions were prepared within an hour of the injection time. Sodium chloride solutions were boiled twenty minutes and made up to volume. Glucose solutions were autoclaved. Weighed portions of supposedly sterile sodium bicarbonate were folded in papers, inserted in a water-tight finger of an old rubber glove, and autoclaved; all cultures were negative. This last procedure was used because some of the bicarbonate heated in solution changes to the more irritating carbonate.¹⁵ The neoarsphenamine was added to the solutions of the vehicle just before injection. This is important, in that, if the sodium bicarbonate increases the rate of oxidation of the neoarsphenamine when it is exposed to air,^{16, 17} there would have been very little time, in our experiments, for such an action to have occurred. Likewise, there could have been but little development of acidity in the glucose solutions.¹⁸ All vehicles were used in strengths corresponding to one-half, one, and two times isotonicity.

Variations in the tonicity and in the concentration of neoarsphenamine (0.1 to 1 per cent), produced no appreciable effect on adhesion formation.

In the glucose series, adhesions were formed in 5 of the 6 dogs and were about the same as those formed when only water was used. One of these, Dog 41, whose death was mainly due to pneumonia, had a small 2 mm per-

TABLE—SUMMARY

ANIMAL		VEHICLE	PERITONEAL PATHOLOGY						PERITONITIS NO	NO PATHOLOGY (PER CENT)	
			ADHESIONS		SLIGHT (PER CENT)		NO	MARKED (PER CENT)			
			NO	(PER CENT)	NO	(PER CENT)					
Guinea Pig	8	Water	0	0	0	0	0	0	0	100	Varying species
Rabbit	10	Water	0	0	0	0	0	0	0	100	
White Rat	6	Water	0	0	0	0	0	0	0	100	
Dog	7	Water	3	71	1	14	0	0	0	14	
Cat	2	Water	2	28	3	43	0	1	1	14	No restraint Dorsal shoulders elevated 20 for 12 hours
Dog	7	Water	5	71	1	14	0	0	0	14	
Dog	6	Water	3	50	1	17	0	0	0	33	Varying vehicle
Dog	7	Water	3	71	1	14	0	0	0	14	
Dog	6	Glucose	4	64	1	17	1	1	1	17	
Dog	3	Ringers	1	33	0	0	0	0	0	0	Varying tonics and H ion All NaHCO ₃
Dog	2	NaCl	0	0	1	50	8	0	0	83	
Dog	12	NaHCO ₃	0	0	1	8	25	0	0	30	
Dog	4	4 Iso	0	0	1	25	0	0	0	100	
Dog	4	2 Iso	0	0	0	0	0	0	0	100	

All Received 0.01 gm of neomphenamine per kilogram of body weight as a 1 per cent solution in 0.1 ml at intervals of four to seven days

foration of the ilium leading to a cavity $\frac{1}{2}$ by 1 mm, with a wall 1 mm thick, backed by adjacent loops of the intestine and its mesentery

Ringer's solution formed a milky precipitate when added to the neoarsphenamine, and 2 out of the 3 animals injected died from a severe chemical peritonitis. The use of this vehicle was not logical, but the result serves to emphasize the toxicity of a cloudy solution of neoarsphenamine.

The sodium chloride series suffered heavily in a pneumonia epidemic. One of the two animals surviving had a single firm adhesion after two injections.

In twelve dogs injected with neoarsphenamine in a sodium bicarbonate solution, only one delicate adhesion (between the omentum and the intestinal mesentery) was found. Such a low incidence is probably well within the normal range for the adult street dogs used. None of the animals showed any of the symptoms ascribed to the oxidized, toxic forms of neoarsphenamine.⁸ One, Dog 76, died six days after the fifth injection from a gangrenous intussusception and peritonitis. This intussusception should be considered since among 19 dogs injected once, and so, not included in the present report, two, receiving water and one per cent sodium bicarbonate as vehicles respectively, died from the same cause. The first, however, had evidently had a piece of glass wedged firmly in its pylorus for some days. Moreover, it would seem that any irritation of the peritoneal surfaces by the neoarsphenamine would tend to decrease rather than increase peristalsis.¹⁹ Possible etiologic factors of intussusception other than the intraperitoneal injection included the mild stimulation of peristalsis during the excretion of arsphenamines,⁸ intestinal worms, present in all three dogs and recognized as an etiologic factor,²⁰ then too, individual factors, since the peritoneal condition of these animals obtained from the dog pound could only be guessed at by their appearance. Injections made into the bowel wall of operated animals produced neither intussusception nor a perforation in any way similar to that found in Dog 41.

It is an open question why sodium bicarbonate should reduce adhesion formation. A physiologic action might be deduced from the very soothing effect similar solutions of sodium bicarbonate have on the nasal and pharyngeal mucous membranes. Changes in the physical state of the semicolloidal neoarsphenamine may have significance. A chemical reaction probably does not occur under the conditions used.

The therapeutic effect of neoarsphenamine may possibly be increased by injecting it intraperitoneally, in that, subcutaneous and intramuscular injections are similarly modified by body colloids, by the rate of absorption, and by their distribution, and are experimentally more parasiticidal than the intravenous.²¹⁻²² Moreover, if oxidation processes are increased by the alkalinity, then by Voegtlin's "arsenoxide theory,"^{23, 24} we might expect some increase from this source. Then, too, possibly such combinations as occur with the peritoneal colloids would be beneficial by preventing the intravascular precipitation of organic arsenicals which Danysz^{21, 22} considers the cause of the nitritoid reactions. That such combinations do not prevent absorption is shown by the clinical results quoted before. We have, moreover, some unpublished experimental results showing arsenic in the blood and lymph after intraperitoneal injection.

CONCLUSIONS

1 One per cent aqueous solutions of neoarsphenamine injected intraperitoneally cause adhesion formation in most dogs and cats, but not in guinea pigs, rabbits, or white rats

2 The addition of 1 to 4 per cent sodium bicarbonate solution greatly reduces the incidence of adhesion formation following intraperitoneal injections of neoarsphenamine in dogs

3 Conclusions which might be drawn from various accidents include the fact that injections into the lower part of the abdomen which cause omental adhesions would probably lead to distress from traction on the transverse colon and stomach, that a cloudy solution of neoarsphenamine should not be injected intraperitoneally that a patient with a possible paralytic ileus should not be injected intraperitoneally that if adhesions are formed obstruction may result, and that an accidental injection into the abdominal wall may cause death

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NOTE ON THE EFFECT OF LEAD ON RAT SARCOMA*

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DURING work on the toxicity of lead compounds, one of the rats developed a spindle cell sarcoma. The point of interest is that the rat was heavily loaded with inorganic lead and developed the sarcoma in the presence of a marked plumbism, from which it ultimately died.

The following intraperitoneal injections were given in the form of lead nitrate (calculated as metallic lead)

First injection (rat approximately three months old) 10 mg per 100 gm body weight

Second injection (two months later) 15 mg per 100 gm body weight

Third injection (five months after previous) 12 mg per 100 gm body weight

The rat died seven months after the last injection and the autopsy showed two small tumors over the fascia, one the size of a pea and the other somewhat larger, apparently at the sites of injection.

The pathologic diagnosis on this tissue was made by Dr W M Copbridge of Watts Hospital, Durham, N C, and confirmed by Dr A A Thibadeau of the State Institute for the Study of Malignant Diseases, at Buffalo, N Y.

Periodic examination of the blood of this animal showed marked polychromatophilia along with anisocytosis, poikilocytosis, achromia and including the appearance of normoblasts, indicating plumbism. Furthermore, some unabsorbed precipitated lead was found in the cavity.

Tissue examination of the liver showed dense adhesions to the diaphragm and a chronic inflammatory process between the lobules. The kidney showed a chronic diffuse nephritis, both glomerular and tubular, the destruction being severe.

The tumor may have been of spontaneous origin or may have been started by irritation due to the injection (other cases indicated the latter). In either case the development in the presence of excessive amounts of lead is noteworthy.

One case, naturally, proves nothing. It is intended to follow up the matter, but as it will take considerable time, this note is presented in the meantime.

*From the Department of Chemistry, Duke University.
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A NOTE ON THE SENSITIVITY OF THE KOLMER AND KAHN TESTS DURING CHILL AND FEVER*

By JOHN H. BLANCON, M.D. and ALINA R. MAYER, PHILADELPHIA, PA.

IT HAS long been known that nonspecific Wassermann positive reactions have been obtained in patients suffering from fevers. Korschinn and Leibfreid¹ report 50 per cent positive reactions in recurrent fever. Liebelberg reports 40 per cent positive reactions in scarlet fever cases. Weil and Braun² report positives in malaria. With the newer and more standardized and sensitive techniques, however, nonspecific positive reactions do not occur so frequently as in the older techniques and the specificity of the modern serologic tests for syphilis is being established. Kolmer³ states that the sera in acute febrile diseases may become somewhat more anticomplementary than usual but with due care in technique these nonspecific positive Wassermann reactions can be avoided.

The treatment of a group of syphilitic patients with fever induced by non-specific protein, offered an excellent opportunity to test the sensitivity of the Kolmer and Kahn tests during the chill and fever and to compare these readings with those taken at normal temperature, to see if the blood shows a tendency to an increased or decreased sensitivity toward positivity in these morbid states.

The quantitative ice box modification of the Wassermann test described by Kolmer,³ and the precipitation test described by Kahn⁴ were used and the principles outlined by each were adhered to.

A group of syphilitic patients at all stages of the disease, whose serologic tests varied from negative to strongly positive were used. Fever was induced in these patients by the intravenous injection of typhoid paratyphoid, streptococcus, colon and gonococcus vaccines. Blood was drawn in sterile dry syringes from the antecubital vein, immediately before the vaccine was intravenously injected, and again about one and a half hours later when a chill occurred. These constitute the chill studies, which amount to 50 pairs of tests taken on 15 patients, i.e. a test at normal temperature and a test during chill in each instance. Likewise, for the fever series, blood was drawn at normal temperature, and again when the temperature reached 103° or above. This amounted to 50 pairs of tests taken on 18 patients. Each pair of specimens was tested at the same time in the same set up, so that variations could not be attributed to technique.

Chill studies and fever studies were performed at different times, inasmuch as we had not decided to do the former until the latter study had been completed.

*From the Department of Dermatology and Syphilology, School of Medicine, University of Pennsylvania, and the Syphilis Clinic of the Hospital of the University of Pennsylvania. Dr. John H. Stokes, Director.

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foration of the ilium leading to a cavity 4 by 1 mm, with a wall 1 mm thick, backed by adjacent loops of the intestine and its mesentery

Ringer's solution formed a milky precipitate when added to the neoarsphenamine, and 2 out of the 3 animals injected died from a severe chemical peritonitis. The use of this vehicle was not logical, but the result serves to emphasize the toxicity of a cloudy solution of neoarsphenamine.

The sodium chloride series suffered heavily in a pneumonia epidemic. One of the two animals surviving had a single firm adhesion after two injections.

In twelve dogs injected with neoarsphenamine in a sodium bicarbonate solution, only one delicate adhesion (between the omentum and the intestinal mesentery) was found. Such a low incidence is probably well within the normal range for the adult street dogs used. None of the animals showed any of the symptoms ascribed to the oxidized, toxic forms of neoarsphenamine.⁵ One, Dog 76, died six days after the fifth injection from a gangrenous intussusception and peritonitis. This intussusception should be considered since among 19 dogs injected once and so, not included in the present report, two, receiving water and one per cent sodium bicarbonate as vehicles respectively, died from the same cause. The first, however, had evidently had a piece of glass wedged firmly in its pylorus for some days. Moreover, it would seem that any irritation of the peritoneal surfaces by the neoarsphenamine would tend to decrease rather than increase peristalsis.¹⁹ Possible etiologic factors of intussusception other than the intraperitoneal injection included the mild stimulation of peristalsis during the excretion of arsphenamines,⁵ intestinal worms, present in all three dogs and recognized as an etiologic factor,²⁰ then too, individual factors, since the peritoneal condition of these animals obtained from the dog pound could only be guessed at by their appearance. Injections made into the bowel wall of operated animals produced neither intussusception nor a perforation in any way similar to that found in Dog 41.

It is an open question why sodium bicarbonate should reduce adhesion formation. A physiologic action might be deduced from the very soothing effect similar solutions of sodium bicarbonate have on the nasal and pharyngeal mucous membranes. Changes in the physical state of the semicolloidal neoarsphenamine may have significance. A chemical reaction probably does not occur under the conditions used.

The therapeutic effect of neoarsphenamine may possibly be increased by injecting it intraperitoneally, in that, subcutaneous and intramuscular injections are similarly modified by body colloids, by the rate of absorption, and by their distribution, and are experimentally more parasitoidal than the intravenous.^{21, 22} Moreover, if oxidation processes are increased by the alkalinity, then by Voegtlin's "arsenoxide theory,"^{23, 24} we might expect some increase from this source. Then, too, possibly such combinations as occur with the peritoneal colloids would be beneficial by preventing the intravascular precipitation of organic arsenicals which Danysz^{25, 26} considers the cause of the nitritoid reactions. That such combinations do not prevent absorption is shown by the clinical results quoted before. We have, moreover, some unpublished experimental results showing arsenic in the blood and lymph after intraperitoneal injection.

Kahn increases from weakly positive to medium positive. In Case 2 the Kolmer increases from medium positive to strongly positive, while the Kahn increases from negative to weakly positive. In Case 3 the Kolmer and Kahn both increase from weakly positive to strongly positive. In Cases 4, 5 and 6 the Kolmer and Kahn both decrease from strongly positive to negative.

Summary of Observations on Chill—The above results show that five or 10 per cent of the Kolmer tests remained unchanged during the chill while the Kahn test varied. Adding this to the 64 per cent of unchanged Kolmer and Kahn tests makes a total of 74 per cent of the Kolmer tests which gave the same reading during chill as at normal temperature. Five or 10 per cent of the Kahn tests remained unchanged during chill making a total of 74 per cent of the Kahn tests which were unaffected by chill.

Where there were variations eight or 16 per cent of the Kolmer tests showed a tendency toward increased positivity during the chill while three or 6 per cent showed a tendency toward negativity. In the Kahn variations four, or 8 per cent showed an increased tendency toward positivity as compared with seven or 14 per cent which showed a tendency toward negativity.

We conclude from the above that chill definitely changes the sensitivity of the Kolmer and Kahn since 26 per cent of both these tests showed variations in that state. The Kolmer reaction showed a tendency to greater positivity during the chill while the Kahn reaction showed about the same tendency in the opposite direction toward negativity.

Fever Group—This group represents 50 Kolmer and Kahn tests taken at normal temperature and again during the height of fever (103° or more). Twenty seven of these pairs or 54 per cent showed no change in the titer or intensity of the Kolmer Wassermann and Kahn tests between normal temperature and fever height whether the test at the outset was weakly positive or strongly positive. Ten of these pairs or 20 per cent which were entirely negative at the outset remained negative during fever height. This makes a total of 37 of these pairs or 74 per cent which gave the same reading throughout the experiment in both tests. Thirteen of the pairs or 26 per cent, showed variation in titer or intensity between that of normal temperature and that of fever height in either Kolmer or Kahn test.

First Group—The following table presents those cases in which the Kolmer remained unchanged while the Kahn varied.

In Cases 1, 2, 3, 4, 5 and 6 the Kolmer remains strongly positive. In Cases 7 and 8 the Kolmer remains negative. In Cases 1 and 2 the Kahn increases from negative to a weakly positive. In Case 3 the Kahn increases

CASE	AT NORMAL TEMPERATURE		AT CHILL	
	KOLMER	KAHN	KOLMER	KAHN
1	44000	000	44000	221
2	44430	000	44430	122
3	44200	233	44200	444
4	44100	443	44100	111
5	44400	444	44400	122
6	44400	444	44400	122
7	00000	333	00000	000
8	00000	333	00000	000

from a medium positive to strongly positive. In Cases 4, 5, and 6 the Kahn decreases from a strongly positive to weakly positive. In Cases 7 and 8 the Kahn decreases from strongly positive to negative.

Second Group—The following table presents those cases in which the Kahn remained unchanged while the Kolmer varied.

AT NORMAL TEMPERATURE			AT FEVER HEIGHT	
CASE	KOLMER	KAHN	KOLMER	KAHN
1	00000	122	00100	122
2	00000	000	21000	000
3	11000	122	11300	122
4	43100	122	00000	122

In Cases 1, 3, and 4 the Kahn remains weakly positive throughout. In Case 2 the Kahn remains negative. In Cases 1 and 2 the Kolmer increases from a negative to weakly positive. In Case 3 the Kolmer increases from a weakly positive to a strongly positive. In Case 4 the Kolmer decreases from a strongly positive to negative.

Third Group—The following table presents the case in which both the Kolmer and Kahn varied.

AT NORMAL TEMPERATURE			AT FEVER HEIGHT	
CASE	KOLMER	KAHN	KOLMER	KAHN
1	43000	333	00000	000

Both Kolmer and Kahn tests decrease from a moderately positive reaction to a negative one.

Summary of Observations on Fever—The above results show that eight, or 16 per cent, of the Kolmers remained unchanged while the Kahn varied. Adding this to the 74 per cent of the tests where neither the Kolmer nor the Kahn varied during fever, makes a total of 90 per cent of the Kolmer tests which gave the same reading at fever height as at normal temperature. Four, or 8 per cent, of the Kahn tests remained unchanged during fever, making a total of 82 per cent of unchanged Kahns when added to the 74 per cent of Kahns which had remained unchanged in both tests during fever.

Three, or 6 per cent, of the Kolmers showed a tendency to increase toward positivity while two, or 4 per cent, showed a tendency toward negativity.

Three, or 6 per cent, of the Kahns increased toward positivity during fever height while six, or 12 per cent, showed a tendency toward negativity.

We conclude from the above that fever does not greatly affect the Kolmer test, there being only 10 per cent which showed variations, that the Kahn test is more greatly affected by fever, there being 18 per cent which showed variations. Where there were variations, the Kolmer test showed a slightly greater tendency toward positivity than negativity in fever. The Kahn test showed quite a definite tendency toward negativity in fever.

Comparing the behavior of the two tests in chill and fever the Kolmer test showed a much greater stability in fever as compared to chill, there being 90 per cent unchanged Kolmers in the former as compared to 74 per cent in

the latter. The Kahns likewise showed a greater stability in fever as compared with chill, there being 82 per cent of unchanged tests in the former as compared with 74 per cent in the latter. The Kolmer test showed a much greater tendency toward positivity in chill, and very little tendency in fever, while the Kahn test showed a marked tendency toward negativity in both chill and fever.

SUMMARY

Two hundred Kolmer and Kahn tests were performed on a group of 33 syphilitic patients receiving fever therapy (nonspecific protein) whose serologic tests on the blood varied from negative to strongly positive reactions. The tests were performed on blood drawn at normal temperature, during the chill and during the height of fever (103° or more). Serologic results in these morbid states were compared with those taken at normal temperature.

During the chill 74 per cent of both the Kolmer and Kahn tests gave the same readings as at normal temperature.

During the fever height, 90 per cent of the Kolmer tests gave the same readings as at normal temperature, while 82 per cent of the Kahn tests gave the same reading as at normal temperature.

In those series which showed variations the Kolmer test showed a tendency toward increased positivity during chill, there being 16 per cent which showed a tendency toward increased positivity as compared to 6 per cent which showed a tendency toward negativity, and very little tendency during fever height, there being 6 per cent which showed a tendency to increase toward positivity as compared with 4 per cent with a tendency toward negativity. The Kahn test showed a tendency toward negativity in both chill and fever, there being 8 per cent which showed a tendency toward positivity as compared with 14 per cent toward negativity in the former, while 6 per cent showed a tendency toward positivity as compared with 12 per cent toward negativity in the latter.

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TOXINS, ANTITOXINS AND DERMAGENS*

By L. G. HADJOPoulos, M.D., and RICHARD BURBANK, M.D., NEW YORK CITY

WHILE studying the relation of skin tests (diphtheria and scarlatina) to complement-fixation reactions, using the respective toxins as antigens, we came to observe certain facts that throw a new light on the nature of toxins as antigens and their serologic relationship to their respective antibodies.

With a very limited knowledge of the chemical nature of toxins, and still less of the antigenic radical in the complex protein structure, we still assume, in accordance with the generally accepted theory, that all proteins, and only proteins, contain the true antigenic (immunogenic) radical. If toxins were true antigens, they should contain a part if not all of the antigenic radical. It is certain that their parenteral introduction gives rise to specific antibodies, called antitoxins, which is also true with antigens in general. It remains, however, to be proved whether or not antitoxins can be considered true antibodies in all respects.

The Relationship of Skin Tests to Complement-Fixation Reaction—Our study was limited to bacterial toxins in diphtheria, scarlatina, and certain streptococcal focal infections. The experimental data as given in Table I, though insufficient for drawing definite conclusions, are nevertheless indicative of the close relationship between the two apparently dissimilar serologic phenomena. In performing the skin tests, we have strictly adhered to the accepted standard methods, using the New York Department of Health products, except in the one instance of the streptococcal toxin which was produced according to the Banzhaf method of 65 per cent alcoholic saturation. The same toxins were used as antigens in the complement fixation reaction. A hundred units of scarlatinal toxin, 0.01 of the L+ dose unit of diphtherial toxin and 0.01 mg. of focal streptococci were used per test. The active serum technique as performed by us was used throughout.

Comments on Table I—Making due allowance for the limited number of experiments, it is nevertheless apparent that there is a close parallelism between the skin sensitization and the complement-fixation tests. The only logical explanation for this phenomenon is the close relationship existing between the skin reagin and the antibody that freely circulates in the system in the above-mentioned pathologic states. Furthermore, toxin complement fixations are strictly specific in scarlet fever and diphtheria, and partly so in focal streptococcal infection because of the heterogeneous nature of the streptococcus species. Lastly, toxin complement fixations are little, if at all, affected by the presence of the Wassermann reagin in the same individual.

Now that a definite relationship has been established between the skin sensitizing reagin and the complement-fixing reagin, it remains to be seen

*From the Department of Laboratories of Beth Israel Hospital, New York.
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TABLE I

THE RELATIONSHIP BETWEEN SKIN TEST AND TOXIN COMPLEMENT FIXATION TEST

	DEGREE TOXIN FIXATION IN COMPLEMENTAL UNITS	SKIN TESTS, DURATION IN DAYS	WASSERMANN REACTION
Scarlatinal Toxin			
1 M	++++	+++	-
2 K	+	±	-
3 S	++++	++	-
4 M ₁	-	-	-
5 St ₁	±	-	-
6 St ₂	++++	+++	-
7 St	++	+	-
Diphtheria Toxin			
1	++	+	++++
2	+	+	-
3	-	-	-
4	-	-	+
Ch	++	++	-
6 GC	++	++	-
Focal Str Toxin			
1 HK	+	+	-
2 PR	++	++	-
3 DB	+	+	-
4 C	++++	+++	-
5 AP	++	++	-
6 CK	+	+	-
7 CC	++++	+++	++++
8 CC	++++	+++	++++
9 D/	++++	++	-
10 AC	+++	+++	-
11 IH	++++	+++	-
12 KK	-	-	-

NOTE The plus system in the case of complement fixation denotes the degree of reaction in terms of fixed complement units. In the case of skin test the duration of reaction in days.

(1) whether this relationship is due to the two reagents being identical or to the coexistence of both (antibodies) in the same patient and (2) what relationship these reagents bear to antitoxins i.e., the artificially produced antibodies against toxin inoculation

TABLE II

IDENTITY OF THE SKIN AND COMPLEMENT FIXING REAGENTS

DATE	CLINICAL HISTORY	TOXIN COMPLEMENT FIXATION	SKIN TESTS
5/ 2/28	Date of onset of tonsillitis		
	Hemolytic streptococcus isolated		
5/ 4/28	Toxin prepared	++++	+++
5/ 8/28	Cheesy foci in tonsils	++++	++++
5/11/28	Tonsillitis subsiding, cough	++	++
5/14/28	Afebrile feeling normal	+	-
5/18/28	No symptoms	++++	++
5/22/28	Tonsillar relapse moderate	++	-
6/15/28	Normal since May 25 no symptoms	++	-

Identity of the Skin and Complement Fixing Reagents.—We have studied during the course of an acute tonsillitis the first aspect of the question. As antigen we used the autogenous toxin prepared from a strain of hemolytic streptococcus isolated from the tonsil of the patient. The results as recorded

chronologically in Table II strongly suggest that both reagents are identical, because the one increases or decreases simultaneously with the other. The complement-fixing reagent, however, seems to be the main source for both reactions as it is more lasting.

On account of this close similarity between the two reagents and owing to the lack of a proper nomenclature, we take the liberty of calling them collectively *dermagens*.

Is the Relation Between Toxin and Antitoxin That of Antigen and Antibody?—Antitoxins are reaction bodies produced artificially by the parenteral introduction of toxins. Dermagens are naturally found in the system of certain individuals predisposed to certain toxopathies, like diphtheria, tetanus, scarlatina, etc. Ontogenetically, antitoxins and dermagens are dissimilar bodies. It is peculiar, however, that the same antigen (the toxin) has two distinct antibodies: the antitoxin directly produced either by experimental inoculation or naturally through disease, and the dermagen, preexisting apparently independently. How the dermagen originates in the system is beyond the scope of this work.

Thus the dermagens behave like true antibodies to their respective toxins. One might suppose that antitoxins are the true antibodies to the toxins as they are directly produced against toxin inoculation. Experimental data, however, do not substantiate this view. As will be seen in Table III, antitoxins cannot fix the complement in the presence of their respective toxins, but react antagonistically to dermagens. A mixture of toxin-dermagen-complement that would naturally result in the binding of complement is prevented from doing so on the addition of antitoxin, as will be seen in Table III.

TABLE III

THE ANTAGONISTIC EFFECT OF ANTITOXIN TO TOXIN COMPLEMENT FIXATIONS

CASES TESTED UNDER FOLLOWING PATHOLOGIC CONDITIONS	TOXIN DERMAGEN COMP. FIXATION	TOXIN DERMAGEN ANTI- TOXIN COMP. FIXATION ANTITOXIN AMOUNTS C C I E R TEST		ANTITOXIN MIXED WITH COMPL. OF EACH SERUM	TOXIN ANTITOXIN COMP. FIXATION
		0.02	0.04		
Scarlatinal Toxin					
1 M ₃	++++	—	—		
2 K	+	—	—	—	
3 S	++++	++	—	—	
4 M ₁	—	—	—		
5 S ₁	±				
6 S ₂	++++				
7 S ₁	++				

The antagonistic effect of antitoxin on dermagen in the toxin dermagen antitoxin complex as illustrated in Table III admits of two possibilities first, that antitoxin acts directly on the sister reagin the dermagen (a view that could not be substantiated experimentally) second that it acts directly on its progenitor, the toxin. If we accept the latter hypothesis we find ourselves in the difficult position of accepting toxins as true antigens. In the whole realm of true antigens there is not an instance where an antibody that is produced by the parenteral introduction of an antigen will antagonize the complement binding property of the latter. This brings us to the last phase of our work, the investigation of the antigenic nature of toxins.

Are Toxins True Antigens?—Our present knowledge of the chemical structure of toxins is very rudimentary. They are present in the globulin fraction of bouillon culture media in which the respective toxogenic microorganism had grown for a number of days. Toxin (exotoxin) is not a part of the bacterial protoplasm but is a catabolic excretory product. The bacterial protoplasm is a true antigen which on parenteral introduction gives rise to a specific complement fixing antibody. The bacterial toxin does not give rise to a complement fixing antibody nevertheless it manifests certain important antigenic characteristics its parenteral introduction gives rise to an antibody the antitoxin which acts antagonistically to the complement fixing property of the toxin antigen. Although not true antigens toxins exhibit an immunophilic antigenic radical capable of binding the complement in the presence of an independent reagin the *dermagen*.

In a previous work¹ we have demonstrated the possibility of splitting true antigens into a protein (immunogenic) and a lipin (immunophilic) radical. The antigenic properties as exhibited by toxins surely are not immunogenic and if anything they are more immunophilic while the unsplit bacterial protein that elaborates the toxin as a metabolic product exhibits both properties as do all true antigens.

As further evidence in this respect we attempted to extract any lipin present in the purified and dried tonsillitic streptococcal toxin to see if such lipin extracts were immunophilic. The result of these tests we give in Table IV.

TABLE IV
THE COMPLEMENT FIXING PROPERTY OF TOXIN LIPINS AND TOXIN RESIDUE

SERIAL NUMBER	WASSERMANN REACTION	WHOLE TOXIN FIXATION	TOXIN LIPIN FIXATION	TOXIN RESIDUE (DELIPINIZED) FIXATION
665	—	—	—	—
666	—	—	—	—
667	—	—	+	—
671	—	+	++	—
672	—	—	—	—
673	—	+	+	—
675	—	—	++	—
676	—	—	+	—
St ₁	—	—	—	—
St ₂	—	+++	++++	±
CC	—	++	++++	±
H	—	—	+	—
KK	—	+	++	—

From the above list we have excluded all Wassermann positive cases to eliminate the possibility of the nonspecific effect of the Wassermann reagent on lipogenic antigens in general. In the test our position is justified in assuming that the lipin fraction of toxins is the cause of complement deviation. The delipinized toxin was shown to be totally lacking in this respect. The comparison of the whole toxin fixations with the lipin and the delipinized residue fixations speaks in favor of this conclusion.

CONCLUSIONS

1 Toxins are partial antigens embodying only the immunophilic fraction. The bacterial protoplasm, on the other hand, is a true antigen with both immunogenic and immunophilic radicals.

2 The parenteral introduction of toxins produces certain reaction bodies, the antitoxins, which, though specific in nature, cannot be reckoned as true antibodies since the interreaction between them and the toxins does not require complement.

3 The true antibody for toxins is a specific reagent freely circulating in the blood of certain toxin-susceptible individuals. The existence of such an antibody can be demonstrated either by means of the complement-fixation reaction or the particular skin tests. For lack of specific terminology we call this reagent the *dermagen* on account of its peculiar dermoactivity.

4 The significance of our last observation is that it provides, by means of the complement fixation reaction, a uniform and more convenient method to the routine skin test in the determination of certain toxopathies.

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6 EAST SEVENTH EIGHTH STREET

A STUDY OF THE TOXICITY OF STRONTIUM AND COMPARISON WITH OTHER CATIONS EMPLOYED IN THE THERAPEUTICS*

By DAVID LOESER AND A. LINCOLN KONWISER, NEW YORK

VULPIAN (1865) is credited with having introduced strontium as a remedy. J. V. Laborde (1890) proved that it was nontoxic and harmless when pure. Since then, strontium has been employed in medicine by oral administration in the place of sodium and potassium cations as a carrier for a number of anions such as bromide, iodide, salicylate, lactate and others. As with potassium salts, the anions are evidently the active therapeutic agents for the reason that both potassium and strontium are only slightly and irregularly absorbed from the digestive tract. This probably accounts for the failure to establish more convincing evidence in the literature as to the therapeutic value of strontium.

Strontium is a divalent in the same chemical group as the alkali earths magnesium, calcium and barium. A number of pharmacologists have studied the effect of strontium on cells, isolated tissues and blood and compared the effects with those exerted by other elements including members of the same group. The impression gained from a research of these reports is that it resembles more nearly the effect of calcium, but that it is weaker. Sollmann¹ states: "This cation resembles calcium and may take its place in some of its characteristic pharmacologic relations, but it is much weaker and also less toxic."

Like calcium, strontium hastens the coagulation of the blood. This is not true of barium or magnesium. It is eliminated by the intestines. The urine contains only traces of strontium when given subcutaneously. It is said to increase the elimination of uric acid and have an effect on bony tissues which are imperfectly calcified. Like calcium, it exerts a digitalis-like action on the heart, but weaker. Paralysis of the heart by quinine and arsenic is said to be neutralized by strontium and calcium.

There have appeared in recent literature, reports of the intravenous injection of strontium salts, particularly of the bromide. These reports indicate its application in the treatment of urticaria and other skin affections, parathyroid tetany, spasmodic asthma, etc., and other conditions indicating the relationship to the effect of calcium.

A review of the literature on strontium reveals a lack of information regarding its action and toxicity when administered intravenously. We have deemed it desirable that experiments be carried on to determine the definite toxicity of strontium and to compare, if possible, the toxicity and tolerance with the elements more commonly employed in therapeutics such as sodium, potassium, magnesium, and calcium. We submit herewith the tabulated results. We employed white rats and the same technique of intravenous injection we described in 1924.

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Table I represents the results of the intravenous injection of strontium bromide in the form of a five per cent solution indicating the doses, grams per kilo weight, equivalent dose for human of 70 kilos and the amount of elemental strontium and bromine grams per kilo weight

TABLE I
TOXICITY TESTS STRONTIUM BROMIDE 5 PER CENT— $\text{SrBr } 6\text{H}_2\text{O}$

RAT NO	DOSE GRAM/K	EQUIV HUMAN DOSE (70K)	WEIGHT OF RAT	C C INJECTED	STRONTIUM g/k	BROMINE g/k	COMMENT
1084	0.250 Gm	17.5 Gm	150 Gm	0.75 c c	0.061 Gm	0.112 Gm	
1085	0.375	26.25	132	1.00	0.092	0.169	
1100	0.450	31.50	135	1.22	0.110	0.202	
1086	0.500	35.00	260	2.60	0.123	0.225	
1096	0.500	35.00	140	1.40	0.123	0.225	Died 45 min
1099	0.500	35.00	115	1.15	0.123	0.225	
1097	0.550	38.50	130	1.43	0.135	0.247	Died 2 min
1098	0.615	43.05	120	1.48	0.151	0.276	Died on table
1087	0.750	52.5	200	3.00	0.185	0.337	Died on table

With the smaller doses, the animals reacted with the same symptoms following the intravenous injection of sodium bromide, becoming stupefied and remaining lethargic for a long time after recovery from the shock of the injection. It will be observed, however, that the toxicity of strontium bromide is greater than that of sodium bromide. White rats tolerate up to 250 gm per kilo of sodium bromide, whereas our maximum tolerated dose is barely 0.5 gm per kilo of the strontium salt. With the larger doses, the rats struggled violently toward the end of the injection, the hind leg becoming paralyzed before death ensued.

In view of the evident sedative effect of the bromide anion, we believed it desirable to ascertain the toxicity of strontium chloride and strontium iodide and arrive, if possible, at results which would clearly indicate the toxicity of strontium cation with due consideration for the effect of the accompanying anions.

Table II represents the results of the intravenous injection of strontium chloride in the form of a 5 per cent solution.

TABLE II
TOXICITY TESTS STRONTIUM CHLORIDE 5 PER CENT— $\text{SrCl } 6\text{H}_2\text{O}$

RAT NO	DOSE GRAM/K	EQUIV HUMAN DOSE (70K)	WEIGHT OF RAT	C C INJECTED	STRONTIUM g/k	CHLORINE g/k	COMMENT
1088	0.250 Gm	17.5 Gm	123 Gm	0.60 c c	0.082 Gm	0.066 Gm	
1089	0.300	21.0	150	0.90	0.098	0.079	
1104	0.300	21.0	135	0.81	0.098	0.079	
1101	0.350	24.5	140	0.98	0.115	0.093	
1103	0.375	26.25	200	1.50	0.123	0.100	Collapsed but recovered
1102	0.425	29.75	145	1.24	0.139	0.113	Died in 2 min
1090	0.500	35.00	205	2.05	0.164	0.132	Died in 15 min
1091	0.625	43.75	138	1.73	0.205	0.165	Died in 3 min

These rats exhibited much more toxic symptoms. Their struggles were much more violent, great difficulty in breathing and violent twisting of tail before final effect of lethal dose. Greatest tolerance is approximately 0.350 gm per kilo.

Table III represents the results of the intravenous injection of strontium iodide in the form of a 5 per cent solution

TABLE III
TOXICITY TESTS STRONTIUM IODIDE 5 PER CENT— $\text{SrI}_2 \cdot 6\text{H}_2\text{O}$

RAT NO	DOSE GRAM/K	EQUIV HUMAN DOSE (70K)	WEIGHT OF RAT	CC INJECTED	STRONTIUM g/K	IODINE g/K	COMMENT
1092	0.10 gm	17.5 gm	1.5 gm	0.6 cc	0.040 gm	0.141 gm	No visible effect
1093	0.37	26.25	130	0.07	0.04	0.211	
1101	0.425	29.75	110	0.04	0.084	0.240	
1094	0.500	35.00	100	1.00	0.098	0.282	Died 60 hr
1106	0.500	35.00	11	1.4	0.098	0.282	
1108	0.550	38.50	130	1.43	0.108	0.310	
110	0.625	43.75	110	1.38	0.123	0.32	Died 10 min
1095	0.62	43.7	110	1.38	0.123	0.32	Very toxic

The rats did not struggle during the injection and as a whole the solution appeared to be least immediately toxic. Rats which survived regained their previous weight within one week and returned their weight for another week while under observation. The minimum lethal dose of strontium iodide is apparently 0.625 gm per kilo.

Table IV presents for comparison the approximate minimum lethal dose of strontium bromide, strontium chloride and strontium iodide, the equivalent strontium per kilo and the equivalent haloids per kilo, their atomic weight and the ratio of atomic weights to lethal dose.

TABLE IV
COMPARISON OF MINIMUM LETHAL DOSE

	$\text{SrBr} \cdot 6\text{H}_2\text{O}$	$\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$	$\text{SrI}_2 \cdot 6\text{H}_2\text{O}$
Dose per kilo	0.1 gm	0.375 Gm	0.625 Gm
Strontium per kilo	0.123	0.123	0.123
Haloid per kilo	0.25	0.100	0.352
Atomic weight of halogen in each figure	80	35.5	127
Ratio of atomic weights halogens	2.5	1	3.55
Ratio of lethal dose haloid	2.5	1	3.52

A comparison of the approximate minimum lethal dose established very definitely that the toxicity of strontium bromide, chloride and iodide is essentially dependent upon the strontium content. In all three instances the strontium per kilo weight is 0.123 gm. This is further confirmed by the fact that the haloid constituents at the minimum lethal dose are in the ratio of their atomic weights.

Table V indicates the minimum lethal dose of sodium bromide, chloride and iodide.

TABLE V
MINIMUM LETHAL DOSES OF SODIUM SALTS (10 PER CENT SOLUTIONS USED)

	NaBr	NaCl	NaI
Dose per kilo	25 gm	18 gm	13 gm
Dose haloid per kilo	194 gm	108 gm	110 gm

Comparing the minimum lethal dose of the respective salts of sodium and strontium we find

On a $6H_2O$ basis

Strontium Bromide is 5 times more toxic than sodium bromide

Strontium Chloride is 4.8 times more toxic than sodium chloride

Strontium Iodide is 2.08 times more toxic than sodium iodide

On an anhydrous basis

Strontium Bromide is 7.2 times more toxic than sodium bromide

Strontium Chloride is 8.1 times more toxic than sodium chloride

Strontium Iodide is 3.0 times more toxic than sodium iodide

In a previous paper,² we drew attention to the relative toxicity of potassium as compared to sodium as deciding our choice of sodium iodide for the intravenous administration of massive doses of iodides. In this instance, we employed a 10 per cent weight to volume solution as the solution employed clinically was of that concentration. For a comparison of the toxicity of strontium to that of potassium, we chose the chlorides and continued the choice of the chloride for comparison with the elements in the same group. We maintained a concentration of 5 per cent except in the case of magnesium and of barium which exception will be noted later.

We submit Tables VI, VII, VIII, and IX as indicating the toxicity of potassium, magnesium, calcium, and barium.

TABLE VI
TOXICITY TESTS POTASSIUM CHLORIDE 5 PER CENT—KCl (0.67 N)

NO RATS INJECTED	CC PER 100 GM	GM KCL PER KILO	POTASSIUM G/K	CHLORINE G/K	COMMENT
4	0.120	0.060	0.031	0.029	Fairly well tolerated
1	0.150	0.075	0.039	0.036	Very sick
3	0.200	0.100	0.052	0.048	All died within 5 minutes

The minimum lethal dose is approximately 0.090 gm KCl per kilo

Lower doses produced symptoms of extreme agitation or uneasiness in rats after release from board. Toxic doses produced convulsive contortions, then coma, noted slight twitching of paws, heartbeat irregular for three to five minutes before death ensued.

TABLE VII
TOXICITY TESTS MAGNESIUM CHLORIDE ($MgCl_2 \cdot 6H_2O$ 2.06 PER CENT) (0.2025 N)

RAT NO	CC IN JECTED PER 100 GM	GM $MgCl_2 \cdot 6H_2O$ KILO	MAGNESIUM G/K	CHLORINE G/K	COMMENT
1110	0.80	0.164	0.019	0.058	Survived
1122	0.80	0.164	0.019	0.058	Survived
1109	0.85	0.176	0.021	0.061	Died within 5 min
1111	0.85	0.176	0.021	0.061	Died within 5 min
1120	0.85	0.176	0.021	0.061	Died within 4 min
1121	0.85	0.176	0.021	0.061	Died within 3 min
1123	0.85	0.176	0.021	0.061	Died within 3 min

This clearly indicated that $MgCl_2 \cdot 6H_2O$ is lethal to white rats in doses of 0.176 gm per kilo. In all fatal cases, the animals passed through the following stages: violent convulsions, twisting of tail, coma, no visible breathing, irregular heartbeat which continued for some time after respiration ceased.

TABLE VIII

TOXICITY TESTS CALCIUM CHLORIDE—5 PER CENT— CaCl_2 (0.001 N)

NO RATS INJECTED	CC PER 100 GM	GM CaCl_2 PER KILO	CALCIUM G/K	CHLORINE G/K	COMMENT
—	0.14	0.07	0.02	0.046	No immediate reaction
4	0.210	0.1	0.04	0.050	Increased respiration Tendency to minor convulsions
5	0.284	0.142	0.051	0.091	Increased respiration Convulsions and toxic symptoms
6	0.322	0.161	0.058	0.103	Very toxic—three im- mediate deaths
7	0.356	0.178	0.064	0.114	Three immediate deaths. Two barely survived

The minimum lethal dose is approximately 0.170 gm CaCl_2 per kilo

At lower doses noted increased respiration. Toxic doses produced convulsive contortions, gasping for breath and then coma for short period.

It will be observed from Table IX that when employing a 5 per cent solution of barium chloride, death ensues rapidly after injecting as small a volume as 0.05 cc per 100 gm, making it practically impossible to secure accurate measurement. We were of necessity compelled to employ a dilution of 0.5 per cent. The results are indicated in Table X.

TABLE IX

TOXICITY TESTS BARIUM CHLORIDE—5 PER CENT— BaCl_2 (0.48 N)

NO RATS INJECTED	CC PER 100 GM	GM BaCl_2 PER KILO	BARIUM G/K	CHLORINE G/K	COMMENT
1	0.20	0.100	0.066	0.034	Died immediately
1	0.15	0.075	0.049	0.026	Died immediately
1	0.10	0.050	0.033	0.017	Died immediately
1	0.05	0.025	0.016	0.009	Died within 30 sec

TABLE X

TOXICITY TESTS BARIUM CHLORIDE—0.5 PER CENT BaCl_2 (0.048 N)

NO RATS INJECTED	CC PER 100 GM	GM BaCl_2 PER KILO	BARIUM G/K	CHLORINE G/K	COMMENT
1	0.40	0.020	0.013	0.007	Died immediately
1	0.30	0.015	0.010	0.005	Survived
1	0.20	0.010	0.007	0.003	Survived

The minimum lethal dose is about 0.020 Gm BaCl_2 per kilo. Since deaths occurred quickly it was difficult to ascertain immediate cause. The dying animals ran the gamut of violent contortions and twisting of tail, and then ceasing of respiration and heartbeat. For a period of one minute after death, there persisted a twitching or tremor of injected leg or lower jaw, other signs of life having disappeared.

We had at the outset determined to confine our comparisons with the cations employed in therapeutics. We were prompted to extend our studies to barium which is rarely used in therapeutics, for the reason that the toxicity of magnesium, calcium, and strontium was in an order inversely to the increase

in atomic weights Barium is generally recognized as the most toxic of the elements of this group and we believe it to be of interest to include it in these comparisons It will be observed that the lowering of toxicity with the increase of atomic weight does not hold as to barium

We submit herewith a summary of the toxicity of the chlorides of sodium, strontium, calcium, potassium, magnesium, and barium, placing them in the order of their increasing toxicity It will be observed that at the concentration we employed, strontium occupies a position next to sodium, being less toxic than the other cations employed in therapeutics

SUMMARY OF TOXICITY TESTS OF CHLORIDES

	APPROX MINIMUM LETHAL DOSE PER KILG ANHYDROUS SALT	CATION	ANION (Cl)
Sodium Chloride (NaCl)	1.08	0.125	0.655
Strontium Chloride (SrCl_2)	0.223	0.123	0.100
Calcium Chloride (CaCl_2)	0.170	0.061	0.109
Potassium Chloride (KCl)	0.090	0.046	0.044
Magnesium Chloride (MgCl_2)	0.052	0.021	0.061
Barium Chloride (BaCl_2)	0.02	0.013	0.007

THE ANTAGONISTIC EFFECT OF STRONTIUM TO MAGNESIUM

In view of the affirmed similarity of strontium action to that of calcium and of the interest recently displayed in the antagonistic effect of calcium on the respiratory depressive action of magnesium, we carried on the following experiments In order to avoid complications by the evident sedative effect of the bromide anion, we selected magnesium chloride and strontium chloride for this purpose

In Table VII, we demonstrated that magnesium chloride ($6\text{H}_2\text{O}$) is lethal in doses of 0.176 gm per kilo In order to test out the effect of strontium, we prepared a similar solution containing in addition strontium chloride on a 1/5 equivalent basis

Table XI indicates the results of the injection of a solution containing magnesium and strontium

TABLE XI

TOXICITY TESTS 2.06 PER CENT MAGNESIUM CHLORIDE ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 0.205 N) 0.55 PER CENT STRONTIUM CHLORIDE ($\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ 0.0405 N)

RAT NO	CC INJECTED	WEIGHT OF RAT	GM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ KILO	GM $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ KILO	COMMENT
1112	1.14	133	0.176	0.047	Survived
1113	1.03	120	0.176	0.047	"
1124	.98	115	0.176	0.047	"
1125	1.28	150	0.176	0.047	"
1126	.98	115	0.176	0.047	"
1114	1.05	110	0.195	0.052	Died 3 min symp- toms of magne- sium alone

While these rats struggled toward the end of the injection, they recovered quickly from the shock and did not go into coma

It will be observed in Table VII that all five rats died when injected with 0.176 gm of magnesium chloride per kilo When 1/5 equivalent of

strontium chloride was injected with the same dose of magnesium chloride, all the rats survived whereas the same equivalent of strontium chloride was incapable of antagonizing the effect of 0.195 magnesium chloride.

Meltzer and Auer³ failed to demonstrate that strontium neutralizes the inhibitory effect of magnesium. These authors, however, employed the subcutaneous method of injection into the rabbit, an animal evidently less sensitive to magnesium. They state, however, that strontium causes a slight improvement of the respiration, but seems rather to aggravate and hasten other inhibitory symptoms due to magnesium especially the paralysis.

To ascertain the effect of a smaller quantity of strontium, we prepared a solution containing 1/10 of equivalent strontium, 2.06 per cent magnesium chloride (0.0205 N) 0.275 per cent strontium chloride (0.0205 N).

At a dose of 0.176 gm magnesium chloride ($MgCl_2$) one rat died within three minutes after going into coma. Another rat struggled violently, passed into coma with irregular breathing and heartbeat finally recovering. It was evident that it requires a larger amount of strontium to neutralize the effect of the lethal dose of magnesium. We have shown, however, that strontium like calcium, in relatively small quantities does exhibit an antagonistic effect to magnesium.

SUMMARY

We have ascertained by intravenous injection into animals that the minimum lethal dose of strontium is approximately 123 milligrams per kilo when the chloride, bromide, and iodide salts are employed.

We have compared the toxicity of strontium with that of sodium, the least toxic of the cations employed in therapeutics. By continuing the comparison with potassium and elements in its own periodic group, we have established that strontium is among the least toxic of the therapeutic cations, occupying the second position in the following order of increasing toxicities: sodium, strontium, calcium, potassium, magnesium, and barium.

In view of the extended clinical administration of strontium bromide and the comparatively low toxicity of strontium demonstrated by these experiments, it is to be concluded that strontium is adaptable for intravenous administration, providing properly controlled solutions and doses are employed.

We have demonstrated also that strontium antagonizes the toxic effect of magnesium by counteracting the respiratory depressive action thus corroborating the impression that the action of strontium resembles that of calcium.

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A NOTE ON BLOOD CHEMISTRY*

BY R S HINT, PH D BOSTON MASS

IN MAY of the current year I completed the chemical analysis of 262 samples of blood taken from members of the last five freshman classes of the Boston University School of Medicine. This work was done as part of the Vital Function Studies described by Rowe¹ and carried out yearly since its inauguration.

Since the subjects from whom the bloods were taken are on the whole, normal young men and women (chiefly men) of an average age of twenty-three years, living the life of the average college student the figures obtained may be of interest and are given in Table I. All bloods were taken after a fasting period of at least twelve hours' duration.

TABLE I

AVERAGED VALUES EXPRESSED IN MG PER 100 CC OF WHOLE BLOOD†

YEAR	NPN	UREA N	URIC ACID	CREATININE	SUGAR
1925	31	16	3.7	14	98
1926	33	16	3.6	14	96
1927	32	16	3.3	13	99
1928	33	16	3.7	14	97
1929	31	15	3.8	15	98
Average (5 yr.)	32	16	3.6	14	98

†Note. The Folin-Wu system of blood analysis was used throughout with the exception of the uric acid determination which was that of Benedict-Franke.

Table II shows the percentage falling within normal limits as defined therein.

TABLE II

NPN	UREA N	URIC ACID	CREATININE	SUGAR
25-35 mg	12-20 mg	2.5-4.0 mg	0.5-1.5 mg	80-120 mg
92.4%	95.8%	82.9%	95.8%	99.6%

TABLE III

	NPN	UREA N	URIC ACID	CREATININE	SUGAR
High	42	28	5.2	20	134
Low	24	10	2.4	11	81

No explanation is offered for the high and low values obtained. The individuals are still in the medical school or are interns in the Hospital and have shown no organic impairment at any time or have other function tests as applied given any evidence of renal impairment.

REFERENCE

- 1 Rowe, A. W. et al. Vital Function Studies II. A Group Study of Certain Blood and Urine Findings, and Gaseous Metabolism, Boston M. & S. J. 192, 747-752, 1925.

*From the Boston University School of Medical and Evans Memorial.
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STUDIES IN THE ALIMENTARY TRACT OF MAN*

III THE GASTRIC RESPONSES TO MILK AND BUTTERMILK

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INTRODUCTION

IN PREVIOUS essays we¹ have dwelt upon the necessity of reducing to a minimum the influence of central stimuli in the gastric reaction pattern before this is employed in experiments upon the human subject. We have shown how the effect of this influence can be quantitatively measured and how by an adequate course of training its disturbance of the gastric response can be rendered negligible. In the present article we shall set forth the results of our experiments on feeding diagnostic meals to stabilized stomachs.

GASTRIC MOTILITY

It is our intention to refrain from entering into discussion of the theory of tone. In our experience tone and peristalsis are both expressions of gastric motility. We acknowledge the clinical pictures of orthotony, hypertony and hypotony but we emphatically deny that these terms are adequate to define gastric tone. For us they imply nothing, whatever regarding the state of tone they are merely conventional phrases indicating differences in the length phase of gastric musculature. Peristalsis is not necessarily less vigorous in hypotony nor more active in hypertony. Both tone and peristalsis are affected by obscure influences which for want of a better term we call psychological. Experiments discussed in our previous articles have separated the effect of this psychological influence from the direct reflex response of the stomach itself.

PERISTALSIS

Immediately upon swallowing the barium meal, our stabilized and trained stomachs show one or more zones of irritability (tension rings) on both curvatures or on the greater curvature alone. After some seconds these zones begin to pulsate and shortly thereafter propagate waves which pass toward the pylorus in orderly sequence and at a rate which shows little if any, variation from one individual to another. In the final phase the pulsating zones disappear as such, and peristaltic waves alone are to be seen. When pulsation appears in the indentations it tends to occur in all at the same time, but peristalsis commences at the zone nearest the pylorus and with each successive wave, originates progressively in the zone nearer the cardia, until the pulsation

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nearest the Magenblase is propagating its wave. We have never seen a preliminary inhibitory phase in advance of the peristaltic wave.

The usual positions of pulsating zones are at the junctions of the three main stomach divisions, gastric tube, pyloric vestibule and pyloric canal, and in the mid-gastric tube. Less frequently we see a pulsating zone in the upper gastric tube just below the Magenblase though this may be due to difficulty of discerning a zone in this relatively faintly outlined area. The size of meal seems to bear a relation to the number of pulsating zones. With a 16-ounce meal we have rarely seen more than one zone, and that at the junction of pyloric canal and vestibule or perhaps in the mid-vestibule. But with a 5-ounce meal we have frequently noted a pulsating zone as high as mid-tube. This observation is probably to be correlated with Trendelenburg's finding that peristaltic activity is stimulated by distension under pressure but that although peristalsis tends to be increased by pressure it rapidly becomes inhibited by over-distension.

Timing the peristaltic waves by stop watches we find speed from particular gastric landmarks remains constant with almost no individual variation. One wave may pulsate longer in the zone of its origin but the time required for its transmission to the pylorus does not change. A wave beginning at the junction of pyloric vestibule and canal takes 7 seconds to reach the pylorus, one beginning in mid-vestibule takes 15 to 18 seconds, if the wave starts at junction of tube and vestibule it takes 32 to 35 seconds to reach the pylorus. With a regular milk meal at 70° F. we very seldom see more than two waves at once in the stomach and the time interval between these is about 20 seconds so that the first is already passing through the pyloric canal as the second encroaches upon the pyloric vestibule. It is not uncommon to see a peristaltic wave which has started in the gastric tube die out on the vestibule and reappear in the pyloric canal, the time interval remaining unchanged. Increase in speed of peristalsis seems to be an illusion produced by the presence of successive waves; it is the time interval between them which is reduced. There is great variation in amplitude of the contraction in different subjects, experiments or conditions. It is diminished, for example, by physical weariness, increased by buttermilk. It varies in amplitude even in the successive divisions of the stomach. Sometimes amplitude is greatest at the origin of the wave in the gastric tube but usually increases as the wave approaches the pylorus.

There is a manifestation of irritability, somewhat resembling an ill-coordinated peristalsis, in which waves of minimal amplitude occur; we have called it shimmer. It usually occurs on the greater curvature alone, though more rarely it is seen equally on both curvatures. No definite orderly progress can be identified in these wavelets but the entire outline of the greater curvature seems to flicker and the shallow wavelets appear in such rapid succession as to give the impression of stationary pulsating minute indentations. In a very active buttermilk stomach where waves are of considerable amplitude it is not uncommon to find shimmer superposed in each large peristaltic wave.

When peristalsis is visible on the lesser curvature it is best seen in the pyloric canal and is then obviously a part of the wave of contraction passing along the greater curvature. When the lesser curvature wave is independent

of that on the greater it seems to have a rhythm of its own. Its amplitude is always less than on the greater curvature. When peristalsis is deep in the pyloric canal it is evident on both curvatures as a circular zone of contraction. We seldom see movement on the lesser curvature proximal to the incisura angularis. The lesser curvature cannot be seen so distinctly as the greater and this may partially account for the scantiness of our information on lesser curvature peristalsis.

Gastric peristalsis invariably ceases at the pylorus. We find no evidence whatever for the view that the wave in the pyloric canal is distinct from that in the rest of the stomach.

In presenting the foregoing summary which is intended to describe the full series of successive phenomena observed in a trained and stabilized stomach, we must make the reservation that these phenomena do not invariably appear in complete expression. They may indeed sometimes pass through successive phases so quickly as to be unrecognizable or apparent only as a fore-shortened sequence with definite phases omitted. Further the description can not be forced to fit the erratic and often bizarre appearances which are to be found in normal but untrained or non stabilized stomachs.

DIAGNOSTIC METHODS

With stabilized trained stomachs as our working material we set out to investigate the effect of barium sulphate, mill and buttermilk on the stomach. On the fluoroscopic screen one has no difficulty in seeing the marked contrast between the effects of mill and buttermilk on the stomach. But as the fluoroscopic appearance does not lend itself to permanent pictorial record observations on area of stomach shadow must be made by radiograms which, in their turn, give merely chance evidence of motility.

The subjects are given a 'dinner meal' consisting of four ounces by volume of mill or buttermilk at a temperature of seventy degrees Fahrenheit, with thirty three grams of barium sulphate. The conditions of examination are constant so that radiograms are exactly comparable.

On the fluoroscopic screen buttermilk is seen to induce immediately gastric elongation and lateral distension with increased amplitude of peristalsis and passage. Sometimes gas in the flexures of the large bowel or in the transverse colon causes indentations which hold up contents temporarily in the cardiac sac. The stomach may be prevented from elongating directly downward. If so, the elongation takes place obliquely so that the greater curvature is swung over to the right of the vertebral column, thus changing the whole shadow outline and giving the illusion of a shorter stomach. In oblique elongation passage is not so clearly seen for the second part of the duodenum is hidden by the distal pyloric vestibule and canal but contents can readily be seen in the jejunum. Typical buttermilk waves are wide and deep, and pass along the entire contents with a regular rhythm.

Milk, on the contrary, usually enters the stomach more slowly than buttermilk. It gradually progresses to the pylorus with relatively little lateral distension or elongation. As soon as the milk contents reach the pylorus a rapid

shimmering movement is set up along the greater curvature in the pyloric vestibule. In some stomachs this is the sole evidence of activity; in others the shimmer gradually becomes deeper until it is transformed into shallow waves. The first wave usually appears in the pyloric canal but succeeding ones start in the progressively proximal sites until in due course they travel the entire length of the stomach. The gradually sinking level of the barium contents during the first ten minutes after ingestion indicates that passage is actually occurring although during this period it can be seen in the milk stomach only by a trained eye to which it is evident as a faint puff.

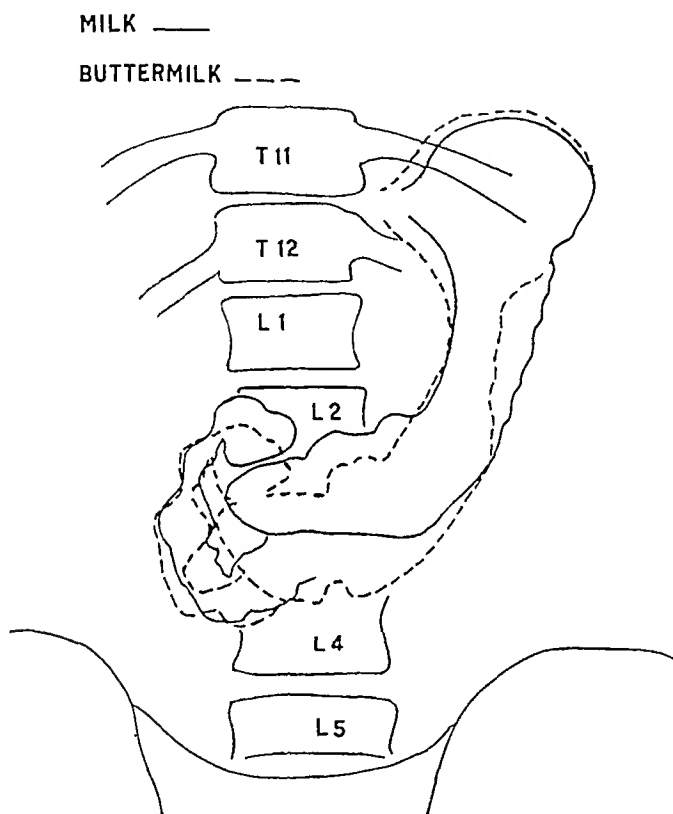


Fig 1—Barium vehicles and stomach outline. Buttermilk is associated with a larger longer usually broader stomach shadow with more vigorous peristalsis. Apparent constriction in buttermilk gastric tube is the imprint of gas in colon on stomach outline.

Barium milk contents are also invisible in the small intestine until the expiration of ten minutes which is the approximate time required for emptying milk *in vitro* at 98° F.

These distinctions of effect between milk and buttermilk meals are observed on the fluoroscopic screen but differences in gastric response thus induced can also be shown quantitatively. Fig 1 illustrates this pictorially and Table I sets forth the distinction statistically. Average gastric shadow-width is 50 mm for both milk and buttermilk, determined immediately below the Magenblase, but the gastric tube itself is distinctly broader after the buttermilk meal. In average vertical length the milk stomach is shorter and its shadow area but 92 per cent of that after buttermilk feeding.

TABLE I

1 COMPARATIVE GASTRIC DIMENSIONS		MILK AND BUTTERMILK SHADOWS		
	NO	AV WIDTH	AV HEIGHT	AV AREA
Milk	31	11 mm	206 mm	11011 sq mm
Buttermilk	31	11	211	12150

2 INFLUENCE OF ORDER OF FEEDING		MILK AND BUTTERMILK		
				AVERAGE AREA
Milk meal first				11372 sq mm
Milk meal following buttermilk				10673 sq mm
Buttermilk meal first				11937 sq mm
Buttermilk meal following milk				12377 sq mm

FACILITATION

To check our general observations recorded above we arranged the series so that half the number of students receive their milk meal first and then buttermilk meal an hour later. With the other half the order is reversed. Knowing that even trained and stable stomachs show a tendency to interference with the gastric reaction by obscure influences in the first examination of our regular two day experimental session we anticipated that a different picture might be obtained in the examinations of the second meal. In the second part of Table I the results of this inquiry are given. The milk meal if given an hour after buttermilk produces a smaller shadow area than if administered first in the series. Buttermilk on the other hand gives a larger shadow when administered second. The characteristic features of outline are then intensified by exhibiting the vehicle after ingestion of a contrasting meal. This observation is so striking that it is well to accumulate all possible confirmatory evidence. Hence we shall take up the problem again after presenting the result of our studies with heat and cold. We give merely a summary of the influence of milk and buttermilk at the moment.

PASSAGE

Just as tone and peristalsis fluctuate independently the one of the other, so also peristalsis and passage are separate expressions of gastric activity. They may occur together or separately and their association is more a coincidence than a co-partnership. Immediate passage often takes place as soon as the food reaches the pylorus and before peristalsis is set up. This initial passage lasts for but a few seconds but the fact of its occurrence even before the zones of irritability begin to pulsate demonstrates its essential independence of peristalsis. Rapid passage occurs in stomachs exhibiting only an occasional peristaltic wave, whereas there may be shimmer or even occasionally, vigorous peristalsis, with no evident passage. We purpose to extend our observations upon this problem but we are convinced that, while the cause of passage may be sought in gastric tone, it is not to be found simply in the peristaltic wave.

Both milk and buttermilk may be accompanied by immediate passage of barium through the pylorus. When this occurs it rarely lasts one minute, during which, however, the passage is usually continuous in contradistinction to the intermittent passage characteristic of later periods. The shadow of

immediate milk passage is light and often seen with difficulty. It may also be a thin stream whereas immediate buttermilk passage occurs in large dense shadows, often moniliform in silhouette though not discontinuous. This phenomenon appears in the notes as "passage in blobs." In the event of there being a residue of the previous contrasting meal in the stomach, ingestion of a new meal of buttermilk usually results in immediate ejection of the milk residuum or most of it. A meal of milk rarely brings about similar ejection of a buttermilk residue.

The ease with which passage is seen depends on the relation of pylorus to the duodenum. If the pyloric canal happens to be disposed in front of the second part of the duodenum observation of actual passage is often impossible. But if the pyloric canal is directed toward the right there are no difficulties in visualizing passage.

Down the second part of the duodenum and round the bend into the horizontal part the shadow can be followed, thin and smoke-like after a milk meal, dense and black and moniliform after buttermilk. The buttermilk-barium shadow more easily defines the cap and lingers in an evanescent cess-pool at the bend between second and third portions. Duodenal peristalsis is easily seen after buttermilk; it is much more rapid than gastric peristalsis but the oscillating character of the shadow is present here as in the stomach. With every wave of peristalsis the cephalad level of the barium shadow shoots upward for an instant before dropping lower. In the second part of the duodenum this upward darting takes the form of streams or "planing particles" which may even lose themselves in the cap shadow and so appear to be rejoining the main mass in the stomach.

There is no necessary relation between passage through the pylorus and gastric peristalsis. We constantly observe passage in the complete absence of peristalsis or we note well-marked peristalsis with no passage whatever. When peristalsis and passage occur in sequence we are inclined to attribute it to coincidence rather than to cause and effect.

In spite of the apparently greater size and density of shadow in buttermilk passage the stomach empties no more quickly than after a milk meal. This is an important observation which is in no way contradictory of the fact that some stomachs empty of milk or buttermilk alike much more rapidly than others. Such rapid-emptying stomachs usually evince evidence of the anxiety complex.

EFFECT OF QUANTITY

If, in place of a five-ounce meal, a sixteen-ounce meal is given, after the initial passage events occur more slowly. Peristalsis does not start after about two minutes on an average in a milk meal or continue without intermission from the beginning in a buttermilk meal. These features characterize the smaller amount. After a sixteen-ounce meal of milk it may be some time before real waves are seen and the buttermilk waves may be at their fastigium some forty minutes after ingestion. Apparently an over-distended organ does not show peristalsis well and it is only after the passage of a considerable volume of contents that the peristaltic wave develops effectively.

THE IRRITABLE STOMACH

It is well known that, in instances of duodenal disease, the stomach shows signs of irritability. We have had the opportunity of examining two definitely atypical stomachs. In one of these subjects there was evidence of occult haemorrhage in the alimentary canal and a clinical diagnosis of duodenal ulcer was definitely made. In the other there was no such diagnosis but the clinical symptoms were those typical of duodenal irritation. Both of these stomachs showed hypermotility equalling milk and buttermilk responses so far as these relate to peristalsis but retaining the usual relative shadow dimensions and area. Both showed a marked reduction in emptying time. The latter of these stomachs we have examined afresh after subsidence of the symptoms and have observed that although the stomach shows an active reaction to milk, it does not overstep the upper range of normal response. Further consideration of this most interesting phase must be left until we have had greater opportunities for observation.

STANDARD ROUTINE

In the practice of internal medicine, roentgenoscopic examination plays an increasingly important part in the diagnosis of gastrointestinal disease. We are not ourselves concerned with such clinical conditions of the stomach as spasm, permanent cicatrization resulting in the hour glass phenomenon, gastric ulcer as evidenced by the Nissen symptom, or definite malformations of the shadow induced by foreign bodies, linitis, plastica and cancer. These, when present, are obvious enough. We are interested however, in the differential diagnosis of gastric response whether evidenced in dimensions or in motility. And we are greatly impressed with the effect of psychological influence upon gastric reactions. Our extended study of the student stomach has been planned with a view to securing greater definition of technique, and a finer discrimination in the diagnosis of functional disorder.

The first important result of our study is the contention that the usual examination of a starved stomach in a patient under considerable mental strain and even apprehension is not calculated to render easy the diagnosis of his disability. We believe that, wherever possible, a gastro intestinal roentgenoscopic examination should be of a more extended type than that at present given, and that the results of two or three successive examinations are necessary for comparison before reaching a decision. We also believe that the conditions of the examination must be very carefully supervised and that no disturbing influence should be permitted to reach the patient at the time of examination.

The examination itself, we believe, should consist of two phases, and that standard five ounce milk and buttermilk meals (four ounces vehicle, 33 gm BaSO_4) should be given successively to a normally fed patient who has not tasted food or drunk water for two hours previously. The contrast between the gastric responses to the stimulation successively of milk and buttermilk will give a fairly definite indication of the gastric reaction pattern.

In our chapter on diagnostic meals we have spoken of cases of gastrointestinal disorder in which the reaction pattern was exaggerated. We have

stated that our experience must be greatly amplified before reaching a definite conclusion on this matter. But we believe that a standard routine for gastrointestinal examination can be successfully evolved upon the lines which have just been laid down.

The reasonableness of this view seems to us apparent from our studies upon the medical students whose willing and loyal cooperation we gratefully acknowledge and without which this initial study and the further investigations which we propose to make could not possibly be undertaken.

SUMMARY

A milk meal results in a radiographic shadow relatively small in linear dimensions and in area. On roentgenoscopic examination the stomach is relatively inactive with peristaltic waves of small amplitude and low frequency. A buttermilk meal of equal volume results in a gastric shadow greater in its linear dimensions and area. But the stomach is more active and peristaltic waves are of greater frequency and amplitude. At the end of twenty minutes after either 5-ounce meal the stomach enters a neutral phase and shows a picture and a record of rather indifferent character so that it may be impossible to decide whether the meal has been of milk or of buttermilk.

These same meals of milk and buttermilk may be employed for diagnostic purposes and enable us to express an opinion upon the normal or abnormal motility of the stomach. But it is necessary to bear in mind that they can be so employed only after the subject has been trained to roentgenoscopic technique by several successive periods of examination.

Exaggeration of milk and buttermilk effects have been noted in definitely unstable stomachs. In these the milk meal is followed by a motility indistinguishable from that of buttermilk though the relative shadow dimensions and area are not modified.

ABSTRACT

In a trained stomach the ingestion of a 5 ounce milk-barium meal is followed by little elongation or lateral distension and by a peristaltic activity so gentle that it may be evinced by nothing more than a shimmer of the shadow outline of greater curvature. Passage occurs in light shadows like a puff of smoke and the duodenal cap may be indefinite in outline.

Under the same circumstances and in like conditions a 5-ounce buttermilk-barium meal is followed by greater elongation and lateral distension of gastric shadow so that the buttermilk shadow area is greater than the milk shadow area of the same stomach. Peristaltic waves are immediate, of considerable amplitude and massive in appearance. Passage occurs in dense moniliform shadows plainly followed through the duodenum.

These facts may be utilized in planning a standard technique of diagnostic meals.

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LABORATORY METHODS

THE CHEMICAL ASPECTS OF INTRAVENOUS GLUCOSE INJECTIONS*

By C. D. INGERSOLL PH.D. WAUKEGAN, ILL.

IN CONJUNCTION with some work on the preparation of glucose for intravenous injection a study has been made of the chemical aspects of the preparation and administration of intravenous glucose solution. Feeling that the results of this study will prove of interest to the medical profession a resume of the various chemical factors involved and the indicated technique is offered herewith.

PREPARATION OF THE SOLUTION

The aqueous solution of glucose is made by dissolving a high grade of glucose in pure distilled water and then sterilizing the solution obtained. It will be noted below that this final sterilization is a preventive measure following the use of sterile ingredients throughout and should not take the place of previous necessary precautions. The procedure is given in progressive steps after each of which appears a short discussion of the chemical points involved. In Table I will be found data for preparing 1 liter (2.2 pounds) glucose solutions of 10 per cent, 20 per cent, 25 per cent and 50 per cent concentrations. The amount of solution prepared at one time may be varied as desired by multiplying the quantity given by an appropriate factor.

MATERIALS AND SOLUTIONS NECESSARY

Glucose C. P.—The glucose should be sterile and of approved high quality. It should contain no starch or dextrose and no *pathologically poisonous salts* such as those of lead or arsenic which are sometimes used in industrial purifications. This also applies to oxalic acid and likewise to carbohydrate polymers of a caramel nature resulting from poorly controlled drying technique in manufacture. Either anhydrous or hydrous glucose may be used if allowance is made in weighing for the molecule of water of crystallization. For purposes of expressing the glucose content of one form in terms of the other, the following equivalents may be noted:

- 1 part by weight of Glucose Anhydrous—1.1 parts by weight of Glucose Hydrate
- 1 part by weight of Glucose Hydrate—0.91 parts by weight of Glucose Anhydrous

Distilled Water—Tap water distilled without proper precautions may and usually does contain varying amounts of ammonia and nitrogenous compounds,

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organic matter, volatile acids, and even salts where the distillation is violent enough to cause entrainment of minute droplets. As traces of these compounds are liable to have a deleterious action on the patient when injected into the blood stream, it is highly desirable that they be eliminated. This may be accomplished by a convenient assembly of two distilling flasks in series. The first flask contains the raw (tap) water and a small amount of potassium permanganate (10 to 15 gm) and the second flask about 100 gm (or approximately 4 ounces) of barium hydroxide. A condenser is connected with the second flask. With the above apparatus chain, all of the organic matter is quickly oxidized in the first flask by the permanganate and for the most part is carried over, as carbon dioxide, through the barium hydroxide solution where it is entrained, together with any volatile acid present. An extremely high-purity sterile water is thus obtained.

It is further to be noted that only freshly distilled water should be used as bacterial growth has been found even in distilled water when stored for any length of time.

Data for intravenous glucose solutions of varying concentrations are given in Table I both in metric and English units and for the use of glucose anhydrous or glucose hydrate. It goes without saying that only glassware that has been washed and rinsed with distilled water should be employed.

TABLE I
GLUCOSE SOLUTION DATA

GLUCOSE CONCENTRATION DESIRED	GLUCOSE, ANHYDROUS				GLUCOSE, HYDRATE			
	METRIC SYSTEM		ENGLISH SYSTEM		METRIC SYSTEM		ENGLISH SYSTEM	
	GLUCOSE	WATER	GLUCOSE	WATER	GLUCOSE	WATER	GLUCOSE	WATER
Per cent	gm	cc	ounce (av)	ounce liq	gm	cc	ounce (av)	ounce liq
10	100	900	3.525	30.42	110	890	3.88	30.10
20	200	800	7.05	27.05	220	780	7.76	26.35
25	250	750	8.82	25.35	275	725	9.70	24.50
30	300	700	10.58	23.65	330	670	11.63	22.65
50	500	500	17.63	16.90	550	450	19.39	15.22

The solution obtained should be clear and brilliant, although a slight sedimentation may occur on standing, the amount of which will depend on the quality of glucose used. There are also possibilities of a few dust particles. The prepared glucose solution should therefore be allowed to stand long enough to complete sedimentation, and then filtered through a high grade of filter paper which is first washed through thoroughly with distilled water. Low quality filter papers are liable to contain a small amount of soluble starch bodies and loose fibers. The washing of filter paper mentioned above is to eliminate possible sources of trouble from this source even in high grade papers.

The P_H should now be tested. Glucose is unstable in alkaline solution (i.e., those having a P_H greater than 7.0). On the other hand, the P_H of the blood stream is 7.3 to 7.4 (just on the alkaline side of neutrality) and is maintained there by the normal blood buffers.

It would appear the better part of good practice to adjust the P_H of the intravenous glucose solution to from 6.5 to 6.8 (just on the acid side of neutrality) using a small amount of sodium carbonate solution, or sodium phosphate

solution if necessary. The blood buffers will quickly absorb this slight acidity and maintain the normal blood P_{H_2} .

Immediately after filtration and adjustment of P_{H_2} , the solution is weighed into clean injection flasks, which have been rinsed with distilled water previous to drying. The flasks are then stoppered with corks that have been boiled in distilled water and the whole capped with a double layer of gauze between two double layers of filter paper. The cap is held on with a rubber band around the flask neck. After autoclaving for thirty minutes at 15 pounds, the flasks are removed, the corks pressed firmly into place and the paper gauze cap secured with several turns of adhesive tape.

The solutions should now be clear and brilliant and contain no foreign matter or insolubles of any kind.

STERILIZATION OF INJECTION APPARATUS

The sterilization of the needle and glass parts of the injection apparatus is a matter of such standard practice that they need not be entered into here. The principal item of interest at this point is the rubber tubing. Rubber tubing is a very convenient form of laboratory equipment but unfortunately carries certain hazards with its use which should be thoroughly understood in order to be adequately taken care of.

Due to the various compositions, textures and characteristics of the rubber tubings on the market only a high grade tubing should be used.

Due to the sulphur content, free tale and other components of the rubber, it should be boiled for fifteen to thirty minutes in 5 to 10 per cent sodium carbonate solution. This will thoroughly scour the rubber and is preferable to caustic soda as it will not be so violent in its action on the rubber. Following this, the tubing should be rinsed thoroughly by allowing a stream of hot distilled water to flow through it. (Tap water should never be used in the cleaning process as it contains many gelatinous and deleterious impurities which may temporarily adhere to the inner surface of the tubing and be later carried into the blood stream by the intravenous solution.)

The next step in washing is due to the fact that alkali has a decided tendency to cling to surfaces. This may be readily observed by dipping the hand in a soda ash solution and noting the difficulty with which the soda is rinsed off under the tap. It is suggested at this point therefore to rinse the tubing with a $\frac{1}{2}$ to 1 per cent solution of hydrochloric acid *c.p.* (made up with distilled water), and then to finally wash out by running distilled water through the tubing for fifteen to twenty minutes (test for completeness of washing with silver nitrate solution).

Other points of importance are the temperature of the glucose solution at the time of injection and the rate of injection. These points are primarily physiologic ones, however and will not be considered here.

OBSERVATIONS ON THE ESTIMATION OF BLOOD SEDIMENTATION
TIME*

BY ROBERT A KILDUIFE † M D , ATLANTIC CITY, N J

THE recent revival of studies of the sedimentation rate of red blood cells has led naturally to a number of modifications of the original Imzenmeier technic, all of which have their advocates

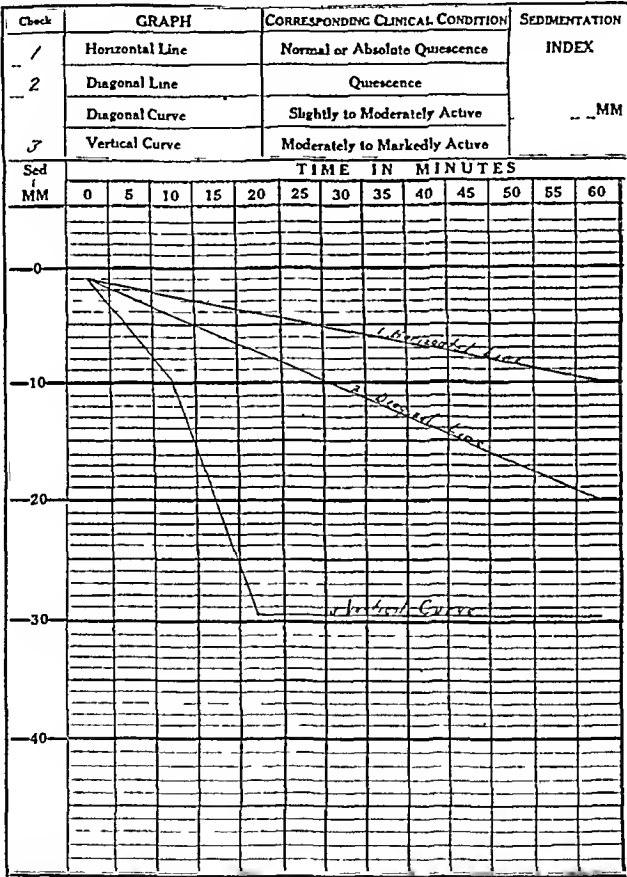


Chart 1

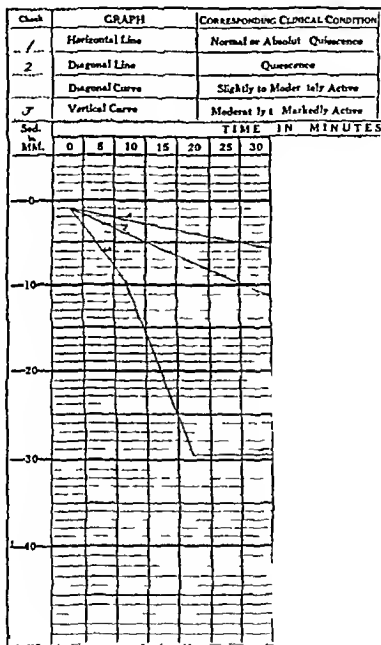
It is more or less axiomatic that the simpler a method can be made without interference with accuracy, the more applicable it will be to the problems of clinical medicine, and many observers, therefore, have endeavored to simplify blood sedimentation technic

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It is not the purpose of this communication to discuss the methods devised for this test but to consider whether or not it is feasible to shorten the time allotted to the reaction and simplify the methods used for reporting the results.

That this is desirable may be hazarded from the multiplicity of methods used at present in reporting the test.

It is commonly accepted that there are two factors of clinical importance (1) the velocity with which sedimentation occurs, which is very closely associated with the rapidity with which it is initiated and (2) the degree to which the cells are finally sedimented.



Chart

Disregarding the variations introduced by variations in technique, it is apparent that the present methods of reporting sedimentation time depend upon the measurement of either distance or time. In other words, either the time is a fixed quantity and the distance through which the cells have fallen during that period is recorded in millimeters or the distance is a fixed quantity, and the time required for the cells to fall that distance is measured in minutes or hours.

As most commonly conducted the reading of sedimentation tests requires a series of observations at short intervals conducted over a period of not less

than one and often several hours, during which the rate or velocity of the sedimentation is recorded at each time interval, the final record being concerned with the total volume occupied by the packed cells. The making of a single test requires, therefore, the undivided attention of the operator for at least one hour.

It is suggested in this communication that this time may be very materially shortened without interfering with the clinical significance of the procedure. The suggestion is based upon practical experience with various methods in several series and upon certain premises immediately following:

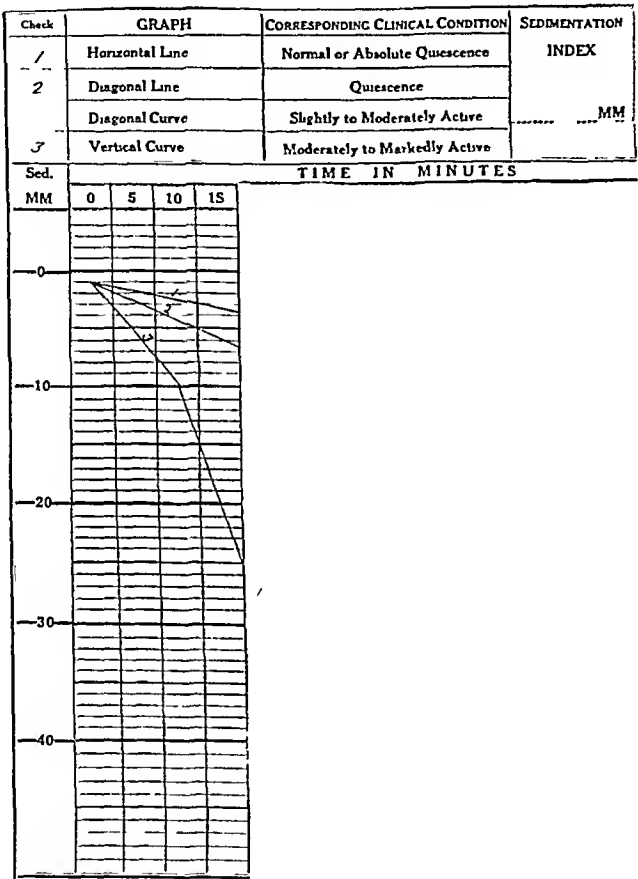


Chart 3

In the first place, while it is not proposed to enter into the debatable field of the exact meaning of the test, it can be said that the majority of observers are agreed upon the fact that it possesses no *differential* value. In other words, while it quite definitely indicates *activity*, it does not definitely distinguish the kind of process which is active.

In the second place, while the velocity rate has been shown to exhibit certain variations when measured at five or ten minute intervals, it is dubious whether these variations are of any real clinical significance. It is true, also,

that regardless of the variations exhibited in any hourly period, the rapidity with which sedimentation begins is related to the velocity with which it continues. It is quite possible, therefore, within fifteen minutes of starting the test to determine whether or not the rate will be within normal limits, rapid, or very rapid.

Finally, there is in general a proportionate relation between the velocity of sedimentation and the final volume of packed cells.

On the basis of these observations, it is suggested therefore, that equally as much information of clinical value can be obtained at the end of fifteen to thirty minutes as can be had after one hour, and that the reading could be reported after the shorter period without detracting from the usefulness of the test.

This may be brought out by comparing the two methods.

The method used in this laboratory is that of Cutler¹ whose charts are also used for report.

In Chart 1 are represented types of sedimentation tests conducted over a period of sixty minutes.

In Chart 2 are shown the same reactions terminated at thirty minutes.

In Chart 3 are shown the same reactions terminated at fifteen minutes.

A comparison of the charts suggests that the final character of the chart at sixty minutes is clearly indicated by the fifteen and thirty minute periods of the same chart. In other words the type of the chart can be seen as easily and clearly at the shorter period as at the end of one hour.

19 6 Cutler J Graphic Presentation of Blood Sedimentation Test Am J Med Sc 86 19

HOT PLATES FOR STAINING SPUTUM SLIDES*

By W D STOVALL, M D AND VERA VINCENT B A MADISON WISC

A HOT plate similar to Fig 1 was adopted for use in the staining of sputum slides for the diagnosis of tuberculosis by this laboratory two years ago. Previous to this time we had been using a copper tank holding 25 slides as in Coplin jars. This was heated by a gas burner. The difficulty of satisfactorily cleaning this type of tank, especially the grooves which held the slides, with the ensuing danger of material from a positive slide being scraped off on the sides of the slots and later becoming attached to a negative slide, brought about a quest for a means of staining a large number of slides quickly with no danger of contamination of one slide by another.

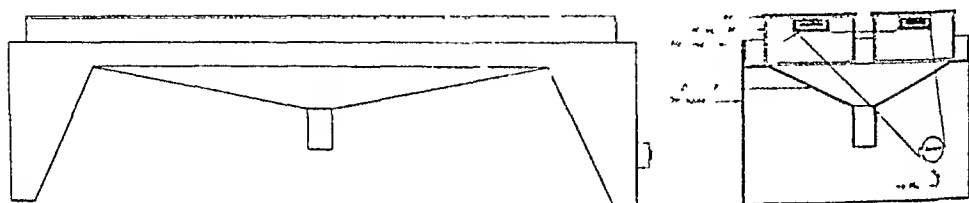
The apparatus developed consists of two monel metal covered heating units each measuring $24\frac{1}{2}$ by $33\frac{3}{4}$ by 2 inches. Monel metal was used to cover the units because it is resistant to the nitric acid in the decolorizer. The heating units proper were purchased from the Westinghouse Electric Company and are designated as Westinghouse Space Heater, S2S4220A 110 volts, 500 watts.

From the State Laboratory of Hygiene Madison Wisconsin

These heaters are 24 inches long, and are covered the full length by the monel metal, which is attached to them on the under side. These two heaters are connected in series in order to achieve the proper temperature for steaming the stain. The current is controlled by a single-way switch attached to the frame of the appliance which can be operated from an ordinary light socket as it uses only 250 watts.

The units rest parallel to each other in a galvanized iron drain pan, the dimensions of which are 26 by 10 inches with $6\frac{1}{2}$ inch legs. The outer edges of the pan are 1 inch deep and slope conically toward a center drain.

We found that the slides, when placed on the smooth monel metal covering the heating units, had a tendency to slip during the washing process and



— 11 IN. PLATES FOR STAINING SLIDES —
7 1/2 1 1/2 1 1/2 1 1/2

Fig. 1

in order to counteract this difficulty two strips of wire screening were fitted over the metal, thereby affording a sufficiently rough surface to prevent any slipping.

The slides are laid on the screen 15 to the unit, which allows approximately $\frac{1}{2}$ inch between slides. They are stained for fifteen minutes, ten minutes with the heat turned on and five after it has been turned off. This gives the slides a chance to cool before the decolorizer is applied, a desirable precaution since we feel that there is a possibility that the slides decolorize too rapidly while hot.

The apparatus is placed near a sink which permits the complete staining and decolorizing process to be carried out on the plates. The stains and decolorizer are applied from dropper bottles permitting a small stream flow of the reagent. A rubber hose, attached to a near-by faucet, is used for washing the slides, and the waste water is carried to the sink by a hose connected to the under drain.

A NEW METHOD FOR THE DETERMINATION OF CHLORIDES IN THE BLOOD*

By T. FOLIN, M.D., AND H. TAUBER, PH.D. BROOKLYN, N. Y.

IN THE following we intend to describe a method for the determination of chlorides in the blood. The method is based on the principle introduced in chemistry by Mohr: the titration of chlorides with silver nitrate in a neutral solution, using potassium bichromate as an indicator.

The necessary solutions are the following: 0.2905 per cent AgNO_3 solution, preserved in a brown bottle; 2.5 per cent Na_2CO_3 solution; 10 per cent $\text{K}_2\text{Cr}_2\text{O}_7$ solution; 1 per cent alcoholic phenolphthalein solution.

All reagents should be especially tested for the presence of chlorides.

Ten c.c. of the protein free blood filtrate prepared in the usual manner according to Folin and Wu, are measured with a pipette in an Erlenmeyer flask. After having added 2 to 3 drops of the phenolphthalein solution the blood filtrate is neutralized, drop by drop with the sodium carbonate solution until a faint red color appears. Three drops of the potassium bichromate solution being used as an indicator at the titration, are now added, whereupon the red color disappears and is replaced by a color similar to that of amber wine. The titration is performed with the silver nitrate solution from a burette. During the titration the blood filtrate gradually assumes the greenish yellow color of lime, and the end point is marked by the changing of this to a brownish color which does not disappear even after vigorous shaking. The change of the colors at the end point is very sharp and a great accuracy of the titration can be secured after a short training.

One c.c. of the above given silver nitrate solution being equivalent to 1 mg. NaCl , the number of c.c. of AgNO_3 solution used in the titration indicates the chlorides present in the blood filtrate as NaCl , in mg. As furthermore 10 c.c. of the protein free blood filtrate represents 1 c.c. of the original blood the multiplication by 100 of the number of c.c. silver nitrate solution consumed during the titration will indicate the chlorides as NaCl in mg. in 100 c.c. blood.

The question as to whether the results obtained by the method described conform with the actual quantity of chlorides present in the blood was examined by two series of checks. In the first series, we measured in each of 6 Erlenmeyer flasks 10 c.c. of protein free filtrate of the same blood. To each of two of these filtrates we added 3 c.c. and to each of other two filtrates 5 c.c. of a NaCl solution of exactly 1 per mille and then we determined by our method the chlorides in all 6 filtrates. The results obtained (calculated for 100 c.c. blood) were as follows: 500 mg. and 493 mg., respectively, in the original filtrates, 797 mg. and 810 mg., respectively, in the filtrates to which 3 mg. of NaCl was given and 998 mg. and 1003 mg., respectively, in the filtrates to

From the Department of Laboratories, United Israel Zion Hospital, Brooklyn, N. Y.
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which 5 mg of NaCl was added. This makes it apparent that we recovered with great accuracy the NaCl added to the filtrates.

In another series, we determined the chlorides in the blood of a number of different patients first by our method and then by Volhard's method as described by Whitehorn,^{*} and compared the results obtained (see Table I).

TABLE I

NO	CHLORIDES AS NaCl, MG IN 100 CC OF BLOOD BY METHOD			
	DESCRIBED		OF WHITEHORN	
	FIGURES OBTAINED	MEAN	FIGURES OBTAINED	MEAN
1	515	516	500	502
	514		505	
	443		447	
2	451	447	433	440
	490		476	
	487		476	
3	558	488	548	476
	565		561	
	530		515	
5	532	531	527	521
	533		533	
	544		525	
6	463	538	469	529
	470		472	
	520		510	
8	515	517	508	509
	475		470	
	482		480	
9	493	478	495	475
	504		494	
	475		463	
10	454	498	458	494
	461		464	
	463		472	
11	530	462	515	468
	539		531	
	508		493	
12	508	534	501	523
	527		510	
	516		515	
13	552	521	550	512
	555		560	
	460		455	
14	460	553	460	555
	592		584	
	606		594	
15	512	460	485	457
	511		495	
	545		540	
16	550	511	545	490
17		547		542
18				
19				
20				

The table shows a great conformity between the results obtained by our method and that of Whitehorn. Taking the latter as a basis for comparison, the differences between the means obtained by the two methods on the same specimen of blood varied between -1.3 and +4.5 per cent. The average figures of all 20 specimens of blood were 509 mg with our method and 503 mg with that of Whitehorn, giving an average difference of +1.2 per cent.

Considering the differences between the two control determinations performed by the same method on each specimen of blood, the highest observed

*Whitehorn J Biol Chem 45 449 1921

difference was 2.3 per cent with our method (No 18) and 3 per cent with Whitehorn's method (No 13). The average difference between the two control determinations was 1 per cent with our method and 1.5 per cent with Whitehorn's method.

The close conformity between the results obtained by our method and that of Whitehorn, but particularly the fact that we recovered exactly the amount of NaCl added to the blood (as shown above) indicates that the method described is one of great accuracy. It offers furthermore distinct advantages over other means of determining chlorides in the blood, one of which is the sharp end point of the titration, as is also indicated by the smallness of the extreme and average difference between the control determinations of the same specimen of blood. The method is also of the greatest simplicity, inasmuch as in contradistinction to other methods it requires only the silver nitrate solution for the titration. Thus the preparation of a second quantitative solution for titration and the frequent painstaking adjustment of the two solutions, as required, for instance, by Whitehorn's method (silver nitrate and potassium sulphocyanate), can be eliminated.

SUMMARY

A method for the determination of chlorides in the blood is described. In addition to an accuracy, comparable to that of the best methods, a distinct advantage of the method described is its greater simplicity.

We wish to express our gratitude to Dr M. Goldzieher, Director of the Laboratories, for his kind interest in this work and for the assistance rendered.

HEATING OF SERA IN THE KAHN TEST*

BY NATHAN NAGLE, A B , AND MARTHA MONELL, ST LOUIS, MO

A STANDARD technic of the Kahn test has been developed as an outgrowth of certain principles which underlie the phenomenon of precipitation in syphilis. Every step in this technic must be rigidly followed to do the test correctly. Any deviation from the method evolved by Kahn, changes the precipitation test sufficiently that it is a modification of the Kahn test and not the standard method. It is unfair to the standard Kahn test when it is performed with antigen that is not standard, with shaking periods varying from the standard with the reading of the test made after overnight incubation, with the serum control omitted and with the inactivation time or temperature changed to meet the mood or fancy of the technician. Each step in the Kahn procedure is vital to the test. Therefore, it is necessary to carry it out in accordance with requirements as prescribed by the author to do justice, both to the test and to the physician and patient.

It is true that the Kahn system has not reached the acme of perfection. It has been devised as a practical test in the laboratory diagnosis of syphilis. As such, it is a very useful aid to the clinician. To the laboratory worker it offers a very convenient medium for studying many problems relating to the serologic diagnosis of syphilis. The comparative simplicity and practicability of the test has stimulated workers in studying the various phases of the precipitation phenomenon. Using the Kahn test in an experimental manner should be encouraged. It should be cautioned, however, that the standard test should be used in all of its details when employed as a routine diagnostic procedure.

One vital step in the Kahn technic is the heating of sera for thirty minutes at 56° C. The end results of the Kahn test are materially changed if any other time or temperature is used. It is possible to change the reaction of a positive serum from four-plus to negative by manipulating the temperature in the heating process. Usually sera giving four-plus reactions with the standard test are negative when the test is made with unheated sera. Negative results may also be obtained if positive sera are heated at 60° C or 62° C for thirty minutes. A change of 2° from the standard temperature over a thirty-minute period may change the reaction of positive sera. Increasing the temperature above 56° C may also account for some atypical reactions. Some sera giving a - + + - - + + + + - + + in the standard test, may give + + + + - + + - -, + - + + + + + + -, or + + + + + - - when the sera are heated at 60° C or 62° C for thirty minutes or less.

Experiments were conducted in this laboratory with the Kahn test on heating sera at various temperatures and for various lengths of time to ascer-

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tain whether it was possible to select a more suitable temperature than 56°C for use in the routine set up of the Kahn test. Experiments were also made to find whether a period shorter than thirty minutes at a temperature higher than 56°C would give good results in this test. In all of this work the standard procedure was always used to check the experimental temperatures and periods of time.

Eighty seven sera were heated at 60°C and 42 at 62°C for thirty minutes and compared to the standard method. A summary of the results is shown in Tables I and II. An analysis of these tables shows clearly that heating sera in this manner tends to inhibit precipitation, especially in sera giving weak reactions. Weak reactions in the Kahn test are very significant in the diagnosis of syphilis and in treated cases as was observed by different workers and in this laboratory.¹

TABLE I

NO OF SERA	8	4	1	1	1	1	1	1	1	1	3	1	63
Standard	++++	++++	++++	++++	++++	+++	+++	++	+	+	+	+	-
56 C-30 min	++++	+++	++	+	-	++	-	+	+	±	-	+	-
60 C-30 min													

TABLE II

NO OF SERA	7	3	1	1	1	1	1	27
Standard	++++	++++	++++	+++	++	+	-	-
56 C-30 min	++++	+++	-	-	±	-	±	-
62 C-30 min								

Judging from the above results it is evident that heating sera at a higher temperature than 56°C for thirty minutes does not improve the Kahn reaction. Accordingly, tests were made on 112 sera heated at 62°C and 175 heated at 60°C for twenty minutes. As shown by Tables III and IV the standard method gives better results.

TABLE III

NO OF SERA	12	3	1	4	1	2	1	2	1	1	1	83
Standard	++++	++++	++++	++++	+++	+++	+++	+++	+++	+	+	-
56 C-30 min	++++	+++	+	-	+++	++	+	-	+	±	-	-
62 C-20 min												

TABLE IV

NO OF SERA	6	2	1	2	1	2	4	1	1	3	1	1	1	150
Standard	++++	++++	++++	+++	+++	+++	+++	++	++	+	+	+	-	-
56 C-30 min	++++	+++	-	++	+	++++	+++	+++	++	+	-	++	±	-
60 C-20 min														

One hundred and thirty eight sera were heated at 62°C for fifteen minutes. Table V shows that these reactions are definitely weaker than the standard test.

TABLE V

NO OF SERA	23	4	1	1	1	2	1	1	1	1	2	100
Standard	++++	++++	++++	+++	+++	+++	+++	++	+	+	±	-
56° C—30 min	++++	+++	-	+++	++	+	-	±	+++	-	-	-
62° C—15 min												

Two hundred and twenty-three specimens were tested after heating at 60° C for fifteen minutes. The results obtained in this group parallel the standard test very closely. Even so, the small number of tests made at this temperature and time is not conclusive evidence that it is more suitable than the standard technic. It would seem that in emergency where the saving of fifteen minutes would be of some importance, it would be safe to use this technic. Table VI shows the results in this series. It will be noticed that even weak reacting sera check very closely with the standard test.

TABLE VI

NO OF SERA	48	3	7	1	1	2	3	1	1	1	155
Standard	++++	++++	+++	+++	++	++	+	±	±	-	-
56° C—30 min	++++	+++	+++	++++	+++	++	+	-	+	±	-
60° C—15 min											

As shown by Table VI heating of sera at 60° C for fifteen minutes approximates the standard method very closely in regard to sensitiveness. It is also seen that heating of sera in this manner is slightly more destructive to the syphilitic reagin than is the standard procedure. Accordingly, 204 sera were heated at 60° C for ten minutes. Even heating sera for this short length of time is sufficient to alter the test so that the reactions are less sensitive than the standard. Table VII gives a comparison of these results.

TABLE VII

NO OF SERA	35	2	1	3	2	1	2	1	1	2	1	1	1	151
Standard	++++	++++	+++	+++	+++	++	++	++	++	+	+	+	+	-
56° C—30 min	++++	+++	+++	+++	++	+++	++	+	-	+	+++	±	-	-
60° C—10 min														

Experiments in which sera were heated at temperatures less than 56° C for different periods of time were not undertaken because of the work done by Kahn. Using unheated sera and portions of the same sera heated at 56° C for five, ten, twenty, thirty and sixty minutes showed that better results were obtained at the thirty-minute period than at the shorter time periods. Heating of sera for sixty minutes resulted in slightly more sensitive reactions than obtained at the thirty-minute period. Other experiments conducted at the same temperature but using longer heating periods showed that the syphilitic reagin began to be destroyed only after ninety minutes of heating. Kahn states, "that the more potent the serum the shorter the inactivation required. The very strongly positive sera apparently did not

require inactivation. Sera of somewhat lesser potency required no more than five minutes of inactivation, while still weaker sera apparently required a minimum of thirty minutes heating."

Sera are heated in the complement fixation tests for the purpose of destroying native complement. Sera are heated in the Kahn test because experiments conducted have shown that this process enhances the precipitation phenomenon. It is not known just what heat does to sera, but it is known that certain temperatures enhance the specific reacting substance while other temperatures destroy this substance. It may be conjectured that heat changes a raw serum from a stable to an unstable form which enables the specific reacting substance in the serum to unite more readily with antigen units. Whatever the explanation may be, it is definitely known that to secure the best results with the Kahn test it is necessary to heat sera at 56°C for thirty minutes.³ Any deviation from this procedure results in a Kahn test that is less accurate and which deviates materially from the standard test.

CONCLUSIONS

The standard heating period of sera for use in the Kahn test is thirty minutes at 56°C . Experiments were conducted to find a more suitable temperature than 56°C and a period shorter than thirty minutes. It was found that heating sera at higher temperatures than 56°C for thirty minutes and for shorter time periods was inhibitory to the precipitation phenomenon and was therefore unsuitable for use in the Kahn test.

This study was aided by the support and helpful suggestions given by Dr. Joseph C. Willett, Chief Bacteriologist.

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RELIABILITY OF THE GONOCOCCUS FIXATION TEST*

BY THOMAS G HULL, PH D, CHESTER GARWOOD, AND NELL HALL,
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THERE are two factors to be considered in the application of the complement-fixation test to gonorrhea, first, from a laboratory standpoint, and second, from a clinical standpoint

Numerous reports of laboratory investigations have found their way into the scientific literature since this test was first used in 1906 During that period considerable progress has been made not only in the sensitivity and reliability of complement-fixation in general, but also in the application of this procedure to gonorrhea when used with a gonococcus antigen

The proper interpretation of the results of the test by the clinician is necessary In some instances unfamiliarity with the shortcomings of the procedure has led to erroneous conclusions, in other cases an undue enthusiasm in favor of or against the test has influenced the interpretation It was our endeavor recently to discover the attitude of clinicians for whom we had been making the test A preliminary survey showed that some had found it, in their experience, reliable and of value to them in determining the diagnosis, others had had an opposite experience while a few seemed loath to say anything against a test in which there might possibly be some good On the whole the attitude of clinicians in Illinois was about the same as that expressed in the "Principles and Suggestions Accepted at the January Conference of Venereal Disease Clinicians" in 1927, "the complement-fixation test for gonorrhea is reported by certain clinicians as a satisfactory diagnostic test, to aid in the diagnosis of chronic gonorrhea especially in cases with suspicious lesions or discharges and negative smears Other reports indicate it is of doubtful value"

The investigation was continued with the results reported in the following paragraphs

THE ANTIGEN

Antigens for the gonococcus fixation test were obtained from four different sources and tested in parallel on the same specimens of serum Antigen C was from a research laboratory, the others were from commercial biologic houses In response to a request for information, the following was submitted as to the method of preparation of each antigen

Antigen A—"The gonococcus antigen is prepared from eleven different strains of the gonococcus from the cultures of which the growth is removed with sterile salt solution and placed in the ice box where it is frequently shaken for a period of twenty-four hours The autolyzed material is then diluted to

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the proper volume with sterile salt solution and preserved with 0.5 per cent thimerosal, after which it is again shaken and placed in the ice box for twenty-four hours before testing."

Antigen B—"The antigen is a bacterial suspension prepared from four Torrey strains."

Antigen C—An autolized extract in normal salt solution made from polyvalent strains of gonococci or a more generalized single strain such as gonococcus No. 34 of Torrey.

Antigen D—"The gonococcus antigen consists of an emulsion of dehydrated, defatted gonococci in 50 per cent glycerol. It is prepared from the two Torrey strains Nos. 34 and 42, which Dr. Torrey recommends as having the best antigenic properties. The antigen is made according to Torrey's method, except that instead of having the finished product in physiologic salt solution, we use 50 per cent glycerol which acts as an excellent preservative and prevents loss of antigenic properties."

The results of the parallel tests are given in Table I. It would seem that the antigen containing the most number of strains of gonococci gave the largest number of fixations.

TABLE I
FOUR ANTIGENS RUN IN PARALLEL ON 100 SERUMS

	ANTIGEN A	ANTIGEN B	ANTIGEN C	ANTIGEN D
Positive	27	6	16	20
Slightly positive	10	3	5	5
Negative	63	90	70	70
Anticomplementary	0	1	0	0

THE TEMPERATURE OF FIXATION

It has been the experience of numerous workers that the ice box method of fixation for a period of four hours to overnight gives the most sensitive results. Comparative tests were made with two antigens by each method. The results, given in Table II, emphasize the greatly increased sensitivity by using the prolonged fixation at 6° to 8° C.

TABLE II
TWO ANTIGENS RUN BY WATER BATH AND ICE BOX FIXATION METHODS ON 74 SPECIMENS

	WATER BATH 30 MINUTES	ICE BOX OVERNIGHT
<i>Antigen A</i> —		
Positive	8	22
Slightly positive	3	2
Negative	63	48
Anticomplementary	0	2
<i>Antigen D</i> —		
Positive	6	14
Slightly positive	5	6
Negative	63	42
Anticomplementary	0	2

THE CLINICAL VALUE OF THE TEST

Clinical histories were submitted with 1170 blood specimens from 822 patients giving age, sex, and clinical diagnosis together with any other pertinent facts. Age was apparently no factor. The specimens were equally divided

TABLE III
THE GOCCOCUS FIXATION TEST IN 822 CASES

	POSITIVE TEST	SLIGHTLY POSITIVE TEST	NEGATIVE TEST
Acute gonorrhea	30	4	26
Subacute gonorrhea	16	2	13
Chronic gonorrhea	63	26	39
Suspected gonorrhea, diagnosis not complete	11	0	0
Old history of gonorrhea	8	0	0
"Cured" cases of gonorrhea	0	2	88
Syphilis	17	3	65
Tuberculosis	1	1	13
Other miscellaneous affections	9	4	90
"Not gonorrhea"	20	15	256
	175	57	590

TABLE IV
RESULTS TABULATED ACCORDING TO SEX

	FIXATION TEST	MALE	FEMALE
Acute gonorrhea	positive	24	6
	slightly positive	4	0
	negative	20	6
Subacute gonorrhea	positive	8	8
	slightly positive	1	1
	negative	7	6
Chronic gonorrhea	positive	40	23
	slightly positive	15	11
	negative	26	13
Suspected gonorrhea (diagnosis not complete)	positive	6	5
Old history of gonorrhea	positive	8	2
"Cured" cases of gonorrhea	slightly positive	2	0
	negative	14	74
Syphilis	positive	11	6
	slightly positive	2	1
	negative	37	28
Tuberculosis	positive	1	0
	slightly positive	0	1
	negative	5	7
Other affections	positive	4	5
	slightly positive	1	3
	negative	44	47
"Not gonorrhea"	positive	13	7
	slightly positive	6	9
	negative	115	135
		414	404

between male and female. Among tests from the 822 patients there were 175 positive reactions, 57 slightly positive and 590 negative (Table III)

Acute Gonorrhoea—Sixty specimens showed 26 negative reactions the majority of which were in the first two weeks of the disease and none later than

TABLE V
INTERVAL BETWEEN DATE OF FIRST SYMPTOMS AND DATE OF TEST

	ACUTE GONORRHOEA		
	POSITIVE TESTS	SLIGHTLY POSITIVE	NEGATIVE
1 week or less	1	0	8
2 weeks	5	0	7
3 "	3	0	2
4 "	3	1	2
5 "	3	0	2
6 "	1	0	1
7 "	0	0	
2 months	2	1	
3 "	1	0	
4 "	1	0	
5 "	1		
6 "	1	0	
8 "	1	0	
9 "	1	1	
1 year	0		
2 years	1		

TABLE VI
INTERVAL BETWEEN FIRST SYMPTOMS AND DATE OF TEST

	SUBACUTE GONORRHOEA		
	POSITIVE TESTS	SLIGHTLY POSITIVE TESTS	NEGATIVE TESTS
1 month or less	1		3
2 months	5	-	1
4 "	1	-	1
6 "	3	-	0
8 "	1	-	1
9 "	1	-	-
10 "	-	1	-
1 year	-	-	1

TABLE VII
INTERVAL BETWEEN DATE OF FIRST SYMPTOMS AND DATE OF TEST

	CHRONIC GONORRHOEA		
	POSITIVE TESTS	SLIGHTLY POSITIVE TESTS	NEGATIVE
1 month or less	1	1	1
2 months	2	0	0
3 "	2	1	2
4 "	1	0	0
5 "	3	0	0
6 "	6	1	3
7 "	0	0	
8 "	0	0	
9 "	1	1	
1 year	10	6	4
2 years	13	1	10
3 "	6	3	4
4 to 7 years	2	0	3
8 to 10 years	3	1	1
11 years and more	2	4	2

the sixth week (Table V) The positive reactions grouped themselves mostly in the second to the sixth week with a few scattered ones later on All but 12 of the specimens from cases of acute gonorrhea were from males (Table IV)

Subacute Gonorrhea—Thirty-one specimens showed 13 negative reactions mostly in the first month The positive reactions on the other hand were mostly in the second to the sixth months (Table VI) The same number of specimens were received from male and female (Table IV)

Chronic Gonorrhea—One hundred thirty-one specimens showed 39 negative reactions The time intervals for most of the specimens were one to eleven years or more after the date of first symptoms (Table VII) There were 81 specimens from males of which 32 per cent were negative and 67 specimens from females of which 19 per cent were negative (Table IV)

"Cured" Cases of Gonorrhea—Ninetv specimens showed 2 slightly positive reactions and the rest negative Many of these were at an institution for women where careful observations had been made over a long period of time Smears and fixation tests that formerly were positive had become negative and remained so consistently

Conditions Other Than Gonorrhea—There were specimens of blood sent in from 494 miscellaneous conditions diagnosed other than gonorrhea Of

TABLE VIII

MISCELLANEOUS CONDITIONS WHICH GAVE NEGATIVE GONOCOCCUS FIXATION REACTIONS

Arthritis	14
Duodenal ulcer	6
Focal infection from tonsil	4
Pregnancy	4
Carcinoma	4
Neurosis	4
Neurasthenia	3
Hypertension	3
Cystitis	3
Hyperthyroidism	3
Nephritis	2
Endocarditis	2
Furunculosis	2
Appendicitis	2
Influenza	2

One each of the following—cardiac hypertrophy, hypotension, polyneuritis tumor, colitis, vaginitis, chronic rhinitis, pyelitis, pyelocystitis, cholecystitis, oophoritis, hemorrhoids, bronchial asthma, aneurysm of aorta, gastritis, chronic bronchitis, acute eczema

TABLE IX

REPEATED NEGATIVE FIXATION TESTS IN 79 FEMALE CASES DIAGNOSED "NOT GONORRHEA"

16 persons	2 negative tests
5 "	3 "
10 "	4 "
9 "	5 "
13 "	6 "
11 "	7 "
5 "	8 "
4 "	9 "
3 "	10 "
2 "	11 "
1 "	12 "

those, 424 were negative, 85 per cent. Syphilis gave 20 positive or slightly positive reactions out of a total of 85 specimens, tuberculosis 2 positive or slightly positive reactions out of 15 other miscellaneous affections gave 13 such reactions as follows rheumatism, neurosis, appendicitis, epilepsy, boils, goiter, sinusitis, hemiplegia one each, pregnancy 2, and adenitis 3. The miscellaneous conditions giving 90 negative reactions are listed in Table IX. There were 291 specimens reported from patients diagnosed "not gonorrhea" of which 6 per cent gave positive reactions, 5 per cent partial reactions, and 89 per cent negative reactions.

The constancy of negative reaction in persons not having gonorrhea is shown in 79 female cases at an institution where two or more tests were repeated on the same individuals (Table IX). Four hundred twenty seven tests repeated on 79 persons gave uniformly negative reactions.

COMMENT

The shortcomings of this form of a statistical study is fully realized. It is difficult enough to obtain accurate diagnosis on a large number of cases at a clinic where the work is carefully supervised. There are bound to be discrepancies, therefore, in diagnoses made by several hundred different physicians some of whom perhaps are not specialists in diagnosing gonorrhea. While the results of the study must be made in general terms they are sufficiently conclusive to be of interest.

SUMMARY

1 The gonococcus fixation test should be made with an antigen composed of as many strains of gonococci as possible. Some antigens on the market contain as few as two strains.

2 The value of the ice box method of fixation overnight is fully confirmed.

3 Cases of acute and subacute gonorrhea show negative reactions early in the disease, but tend to become positive later. Cases of chronic gonorrhea show positive fixation reactions in about 70 per cent of cases.

4 A small percentage of cases which have not been diagnosed gonorrhea give positive reactions.

5 Persons not having gonorrhea that do not give a positive test at one time probably will not give a positive test at a later time. Four hundred and twenty seven tests repeated on 79 individuals were uniformly negative.

REFERENCE

- 1 Report of a Conference of Venereal Disease Control Officers and Clinicians held in New York Jan. 20, 1927, Venereal Disease Information, U. S. Public Health Service 872, 1927.

AN EASILY CONSTRUCTED SLIDE RULE FOR CALCULATING THE DATE ON WHICH A GIVEN INTERVAL IN DAYS WILL FALL

By THEODORE S MOISE, M.D.,* BANGOR, MAINE

THE slide rule, herewith described is an easily made instrument that may be used for saving time and labor in calculating the dates on which a known time interval in days will fall. The author has used it with great benefit in calculating the dates of termination of a large series of experiments, running for definite time intervals.

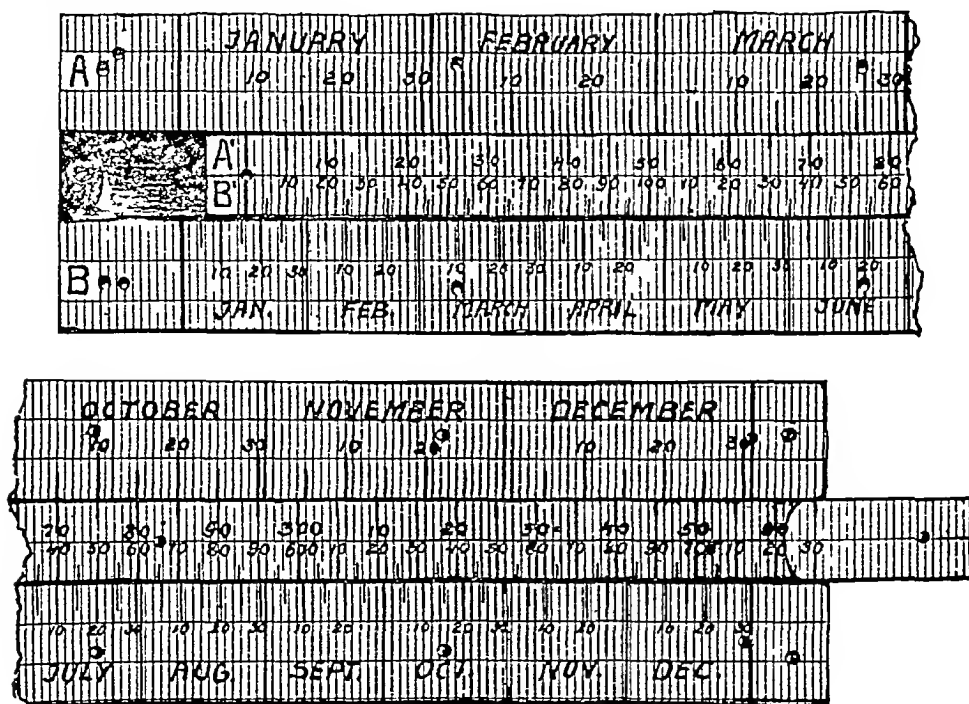


Fig 1

The graduations on the slide rule are measures of length representing increments of time in days. The scales seen in Fig 1 were made on Keuffel and Esser, Standard Profile Paper 4 by 20 to the inch. On scale A, one division ($\frac{1}{20}$ inch) is taken as one day, and a linear calendar† was drawn. The end of each month is represented by lines extending completely across the scale. On scale A' the same unit ($\frac{1}{20}$ inch) represents a day with three hundred and

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†A correction for February 29 (omitted from scales) is easily made when necessary

sixty five days drawn in a linear manner independent of the calendar months. On scales *B* and *B'*, a similar representation of two years is made with each division representing two days. Scales *A'* and *B'* are on the sliding portion of the rule.

A calculation is made in the following manner. Example. Fifty six days after January 8. The zero mark on scale *A'* is set opposite January 8. On scale *A'* (Fig. 1), 56 is then located and the reading March 5 is made above on scale *A*. The rule may be set to make other similar calculations. In practice, scales *A* and *A'* are more useful than scales *B* and *B'* as an accurate reading is more easily made with the larger unit. In calculating the date after an interval extending from the latter part of the year beyond January 1 of the following year, the reading may be made as follows. Example. Fifty six days after December 5. Opposite December 5 on scale *A*, set 365 on scale *A'*.

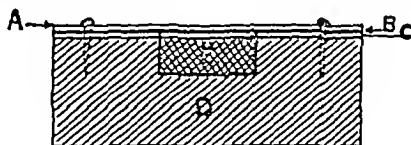


Fig. 1

Turn the eye to the left and read the date on scale *A* just above 56 on scale *A'*. Calculations of more than three hundred and sixty five days may be made on scales *B* and *B'*.

The rule is $\frac{3}{4}$ by 2 by $19\frac{1}{2}$ inches. Scales *A* and *B* are $\frac{3}{4}$ inch wide. The sliding portion of the rule including scales *A'* and *B'* is $\frac{1}{2}$ inch wide. In order to prevent stretching and shrinking of the profile paper (with consequent introduction of an error) the device of mounting* a two inch strip of profile paper on heavy drawing paper was adopted. The scales were drawn on this mounted paper before being cut to the proper size. The scales were fastened to the rule by small tacks. The construction of the rule is shown in Fig. 2. *A* is a thin transparent celluloid cover. *B* is the profile paper on which the scales are drawn. *C* is a piece of heavy drawing paper. *D* is the body and *E* is the sliding portion of the rule.

*Kodak dry mounting tissue was used

A STUDY OF HEMOGLOBIN METHODS*

BY M. KARSHAN, PH.D., AND R. G. FREEMAN, JR., M.D., STATEN ISLAND, N. Y.

MANY clinical methods for the determination of hemoglobin have been proposed. At the present time, with the wide variety of methods in use, many of them of questionable accuracy, it is impossible to compare the results of one worker with those of another or to evaluate the results of a single piece of work because of the inaccuracy of the method employed. The purpose of the work to be described in this paper is to determine the accuracy of a simple clinical method by comparison with a standard method of recognized accuracy.

From the review of Lindsay, Rice and Selinger¹ it appears that of the clinical methods, those of Newcomer² and of Cohen and Smith³ are the most accurate. As for the Talquist, the Dade and Sahli methods, their inaccuracy has been repeatedly pointed out.^{1, 6, 14}

The Newcomer, and the Cohen and Smith methods are based on the same principle, namely, the conversion of a definite quantity of blood to acid hematin by means of dilute hydrochloric acid. The color thus developed is compared with a standard, which in the Newcomer method is a "high transmission yellow" semaphore glass disk. In the Cohen and Smith procedure a standard acid hematin solution is used, and the authors claim an accuracy to within 2 per cent in the hands of inexperienced workers and a margin of error of 1 per cent or less by experienced workers. Shortly after the publication of the method of Cohen and Smith, Robscheit⁴ published a method almost identical to that of the former authors. Comparing this with the Palmer⁵ method excellent checks were obtained.

We chose to investigate the Cohen and Smith method, incorporating the modifications recommended by Oser⁶ in so far as the quantity of blood to be analyzed is concerned. Cohen and Smith use 0.02 cc of blood in 6 cc of 0.1 N HCl, whereas Oser uses 0.05 cc of blood in 10 cc of 0.1 N HCl. A deeper color is thus obtained, and the larger volume makes for greater convenience and accuracy in making the color comparison.

Several attempts were made to prepare the standard hematin solution from oxalated blood. In every instance solutions containing a considerable quantity of a coarse suspension were obtained. This did not occur when the original procedure of Cohen and Smith using defibrinated blood was employed. The standard was prepared by determining the hemoglobin content of the blood by the Van Slyke and Neill⁷ oxygen capacity method and then diluting with 0.1 N HCl to give a 3 per cent solution of acid hematin.† From this stock

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†If the hemoglobin concentration is 14.2 per cent dilute $\frac{20 \times 15}{14.2}$ or 21.1 cc to 100 cc with 0.1 N HCl.

solution, a dilute acid hematin solution was prepared for color comparison with the unknown, by diluting 5 cc to 200 cc with 0.1 N HCl.⁵

The Van Slyke^{7, 9, 1} oxygen capacity methods, while standard, present many technical difficulties and necessitate considerable practice. Wong^{10, 11} has devised a comparatively simple colorimetric method by which hemoglobin is calculated from the iron content. He found that this procedure checked very closely with the Palmer² method for determining hemoglobin. Lindsay, Rice and Selinger¹ report a number of analyses comparing Wong's original method¹⁰ with the Van Slyke¹ oxygen capacity method. On the basis of seven determinations, an average variation of 2.6 per cent was obtained. They recommend the Wong method as a substitute for the Van Slyke method in standardizing hemoglobin solutions.

We felt that a longer series should be obtained before such recommendation could be made, and in obtaining such a series we determined the oxygen capacity of the blood by the manometric method of Van Slyke and Neill⁹ incorporating the recommendations recently made¹² and compared the calculated amount of hemoglobin with the hemoglobin calculated from iron by the method

TABLE I

SPECIMEN	VAN SLYKE PER CENT	WONG PER CENT	ACID HEMATIN PFR. OF T.	
			VAN	FINGER
1	12.9	13.1	12.6	
2	11.5	11.2	11.3	
3	15.3	15.7	15.1	
4	13.1	13.1	13.4	
5	13.2	13.4	13.0	
6	15.7	15.7	15.7	
7	14.3	15.1	14.5	
8	11.2	11.3	10.7	
9	14.0	14.2	13.8	13.4
10	14.3	14.0	14.1	13.8
11	11.5	11.5	11.2	11.3
12	12.7	12.6	12.5	12.2
13	12.8	12.9	12.4	12.5
14	12.8	12.8	12.3	12.7
15	14.4	14.5	14.0	14.2
16	10.5	10.3	10.2	10.2
17	12.6	12.0	13.2	12.9
18	13.2	13.6	13.3	13.2
19	11.7	11.9	11.3	11.2
20	13.5	13.7	13.8	13.5
21	12.5	12.6	12.3	12.4
22	12.1	12.3	11.6	11.7
23	12.4	12.4	11.9	12.1
24	12.3	12.0	12.8	12.5
25	13.0	13.1	13.7	13.4
26	13.0	13.3	13.0	12.6
27	13.6	13.7	13.1	13.2
28	9.8	10.0	9.7	9.5
29	9.3	9.2	9.4	9.5
30	10.0	10.1	9.8	10.0
31	12.9	13.2	12.6	12.5
32	14.7	14.5	14.1	14.4
33	12.1	12.4	12.4	12.2
34	10.7	10.9	10.5	10.5
35	13.3	13.5	13.4	13.3
36	15.7	15.9	15.2	15.4

fied method of Wong¹¹ Each specimen of blood was also analyzed by the acid hematin method of Cohen and Smith and in most cases further comparison was made by an additional determination on blood from the finger (taken immediately after phlebotomy) by the acid hematin method The standard solution was checked repeatedly by fresh standards

The following results were obtained in a study of 36 specimens, taken from male children between the ages of six and thirteen years

In Table I the figures represent the number of grams of hemoglobin per 100 cc of blood (percentage)

It is obvious from Tables I and II that there is a tendency for the acid hematin method to give slightly lower values than either the Wong or Van Slyke procedures, and to differ slightly more from the former than from the latter

TABLE II
THE VARIATIONS BETWEEN THE DIFFERENT METHODS

SPECIMEN	PERCENTAGE OF VARIATION		
	WONG METHOD FROM VAN SLYKE METHOD	ACID HEMATIN METHOD (VEIN) FROM VAN SLYKE METHOD	ACID HEMATIN METHOD (VEIN) FROM WONG METHOD
1	1.55	2.33	3.82
2	2.61	1.74	0.89
3	2.61	1.31	3.82
4	0.00	2.29	2.29
5	1.52	1.52	2.99
6	0.00	0.00	0.00
7	1.34	2.68	3.97
8	0.89	4.46	5.31
9	1.43	1.43	2.82
10	2.10	1.40	3.57
11	0.00	2.61	2.61
12	0.79	1.57	0.79
13	0.78	3.12	3.88
14	0.00	3.91	3.91
15	0.69	2.78	3.45
16	1.90	2.86	0.97
17	2.38	4.76	2.33
18	3.03	0.76	2.21
19	1.71	3.42	5.04
20	1.48	2.22	0.73
21	0.80	1.60	2.38
22	1.65	4.13	5.69
23	0.00	4.03	4.03
24	2.44	4.07	6.67
25	0.77	5.38	4.58
26	2.31	0.00	2.26
27	0.74	3.68	4.38
28	2.04	1.02	3.00
29	1.08	1.08	2.17
30	1.00	2.00	2.97
31	2.33	2.33	4.55
32	1.36	4.08	2.76
33	2.48	2.48	0.00
34	1.87	1.87	3.67
35	1.50	0.75	0.74
36	1.27	3.18	4.40
Average	1.40	2.47	3.05
Maximum	3.03	5.38	6.67

Davis and Sheard¹ in a study of 15 specimens in which the Van Slyke method was compared with the Cohen and Smith acid hematin procedure, found an average variation of 2.5 per cent. This is of the same order of magnitude as the percentage variation obtained by us. Their maximum variation of 7.7 per cent, is, however, a little more than 2 per cent greater than ours.

TABLE III

METHOD	MEANS	DIFFERENCE	PROBABLE ERROR
Van Slyke	12.57		
Wong	12.65	0.10	0.2685
Van Slyke	12.57		
Acid Hematin (Finger)	12.35	0.20	0.2652
Van Slyke	12.55		
Acid Hematin (Vein)	12.45	0.10	0.2677
Wong	12.67		
Acid Hematin (Finger)	12.35	0.30	0.2662
Wong	12.6		
Acid Hematin (Vein)	12.47	0.20	0.2688

It will be seen from Table III that in every case the difference is less than three times the probable error and is therefore not significant.

TABLE IV

CORRELATION OF METHODS BY SPERMAN RANK—ORDER FORMULA

Van Slyke and Wong	0.99
Van Slyke and Acid Hematin (Vein)	0.95
Van Slyke and Acid Hematin (Finger)	0.97
Wong and Acid Hematin (Vein)	0.95
Wong and Acid Hematin (Finger)	0.96

SUMMARY

The modified method of Wong in which hemoglobin is calculated from the iron content of the blood checked very closely with the oxygen capacity method of Van Slyke and Neill, and can therefore be used instead of the latter method in determining hemoglobin for the preparation of standard acid hematin solutions.

The acid hematin method of Cohen and Smith for the determination of hemoglobin was compared with the Wong and Van Slyke and Neill procedures. Good checks were obtained. The simplicity and accuracy of this method warrants its use as a good clinical method. The fact that the standard acid hematin solution needed in this method can be prepared by the comparatively simple Wong procedure, makes it of general applicability.

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A RAPID METHOD FOR DETERMINING THE TYPE OF PNEUMOCOCCUS IN SPUTUM*

BY L. ROSENTHAL, M.D., AND BERNARD STERNBERG, M.D., BROOKLYN, N. Y.

OUR method for determining the type of pneumococcus in sputum is performed in the following way

1 *The Homogenization of the Sputum*—The homogenizing fluid is a combination of two components a borax solution (Stroheim¹) and hydrogen peroxide proposed by Soigo,² and Sachs-Mucke³ in the diagnosis of tuberculosis

One to 3 cc of sputum are placed in a test tube. To this is added borax-boracic acid solution (borax 15 gm, boracic acid 15 gm, water 100 cc) a few drops at a time, until homogenization occurs. The total amount of the borax solution required varies with the character of the sputum. As a rule a quantity equal to the volume of the sputum is used. Homogenization is then completed by the addition of 3 per cent hydrogen peroxide. In most cases $\frac{1}{4}$ to $\frac{1}{2}$ cc of the latter is sufficient, if necessary, more may be added without danger of interfering with the reaction. After the addition of the peroxide the mixture is agitated either by means of a wooden applicator stick or by shaking. In adding the various solutions, it is essential to keep the total volume as low as possible in order to procure the antigen in a concentrated form.

2 *The Preparation of the Pneumococcus Antigen*—The tube containing the homogenized sputum is centrifugalized for two or three minutes at high speed. The mixture then divides into three layers a varying amount of solid material is drawn to the bottom of the tube, a foamy pellicle forms on the surface, and between these there is a zone of fluid. This fluid may be clear and transparent or it may present various degrees of opalescence. It may be aspirated by a Pasteur pipette or poured through the pellicle into a small test tube and recentrifugalized for a few seconds.

3 *The Typing*—The following material is needed (a) Pneumococcus antisera Type I, II, and III (b) A wet chamber prepared by covering the bottom of a Petri dish with a piece of moist filter paper. On the filter paper two wooden applicator sticks of proper length are placed to support the glass slides (c) Clean glass slides divided by a pencil into three sections.

A drop of antipneumococcus serum Type I is placed in the first, a drop of Type II serum in the second, and a drop of Type III serum in the third section of the slide and a drop of the antigen is added to each. The drops of

*From the Laboratories of the United Israel Zion Hospital Brooklyn N. Y.

antigen and serum are carefully mixed with a glass rod. Care must be exercised to use a separate pipette and rod for each type. The slide is then placed in the wet chamber. It may be left at room temperature, but the reaction is hastened by keeping the chamber in an incubator at 37° C. The slides are examined at ten minute intervals for flocculation which may occur in a few seconds or may be delayed from ten minutes to an hour. The reaction is usually observed under the low power of the microscope but it may become so marked as to be visible to the naked eye in the proper reflection of light. The reaction that occurs with the antigen from a Type III pneumococcus sometimes varies from the usual flocculation by the appearance of mucous threads which gradually become more prominent. In some instances the antigenic fluid cannot be entirely cleared of cells and debris, but their presence does not influence the reaction and the flocculi can be easily seen between the cells. The occurrence of flocculation with one of the sera indicates the



Fig 1—Flocculation in homogenized sputum mixed with corresponding antiserum (wet preparation. Zeiss Objective 1. Ocular 10x)

corresponding type. The absence of flocculation with all the three may either indicate Type IV or it may be due to the insufficiency of antigen I, II, or III in the sputum. In nearly all of our cases the absence of the reaction was due to the fact that the sputum contained Type IV organisms.

In order to establish the nature of the flocculation, the following examination was done:

A mixture of a drop of homogenized pneumococcus sputum and a drop of the corresponding serum was allowed to dry on a slide. The film was fixed with methyl alcohol and then stained with methylene blue and examined microscopically under the high power. In most cases we found precipitated granular flocculi (Fig 1) but at times we could also see masses of agglutinated pneumococci (Fig 2). The reaction must therefore be regarded as a combined agglutination-precipitation test.

We typed by our method 81 sputa from patients with lobar pneumonia which we obtained during the months from October, 1928 to February, 1929.

from the various hospitals (United Israel Zion, Beth Moses, Greenpoint, Crown Heights) Every specimen was checked by the mouse inoculation method

Seventy-four sputa showed identical results in both methods, namely 15 cases Type I, 16 cases Type II, 12 cases Type III, 27 cases Type IV, 4 cases Types II and III

Four sputa gave flocculation with all three undiluted sera In these cases we repeated the test using diluted sera in order to delineate the reaction more clearly Dilution was performed as follows

Type I serum diluted 1:10 by addition of one drop of serum to 9 drops of saline,

Type II serum diluted 1:10 in the same manner,

Type III serum diluted 1:5 by adding 2 drops of serum to 8 drops of saline

All four sputa when tested with the diluted sera, reacted according to their respective types, viz 2 cases Type I, 2 cases Type IV

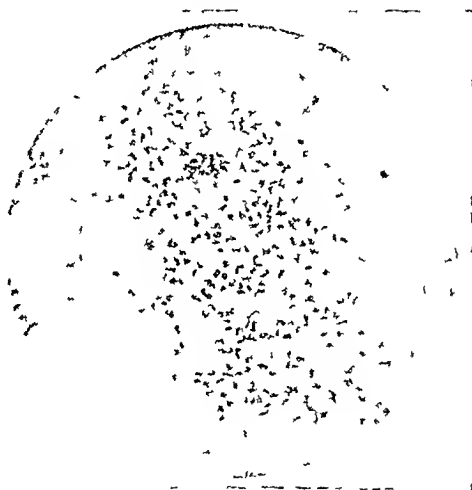


Fig 2—Agglutinated pneumococci in homogenized sputum mixed with corresponding antiserum (Zeiss Oil Immersion Objective 10x)

Only 3 sputa showed a discrepancy between the mouse inoculation method and our method, viz

<i>Mouse method</i>	<i>Homogenization method</i>
Two cases Type I	No flocculation with any of
One case Type II	the sera

The discrepancy may be explained by the assumption that these sputa did not contain a sufficient amount of antigen to be detected by our test

We also typed by our homogenization micromethod pneumococcus pus from nine cases of empyema with the following results 4 cases Type I, 2 cases Type II, 1 case Type III, 2 cases Type IV These findings were confirmed by typing the cultures obtained from the pus It is necessary to add that results were positive in 5 cases not only by the homogenization procedure but also by direct typing of the liquid part of the untreated pus In the other four cases homogenization of the pus was required to establish the type

We also applied our method to the residua of 10 sputa which are discarded as useless after extraction of the antigen by the method of Krumwiede and Valentine⁴ and Ohver⁵. We found that these residua still contained sufficient antigen which could be demonstrated by flocculation with the corresponding serum.

CONCLUSIONS

The homogenization micromethod for the determination of the type of pneumococcus in sputum, pus, etc., fully described above, has the following advantages:

- 1 It allows the direct typing of the material without the use of animals or culturing methods
- 2 It makes possible the determination of the type in a short time
- 3 It requires relatively small quantities of material
- 4 It permits more complete utilization of the pneumococcus antigen contained in the sputum

The reliability of our procedure is vouchsafed by the nearly full coincidence of its findings with the results of the mouse inoculation method.

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A METHOD OF USING WRIGHT'S STAIN IN STAINING JARS*

By ROY F. FEEMSTER, MD, DPH, NEW ORLEANS, LA

THERE has been a long felt want of a method by which blood smears and other preparations might be successfully stained in quantity by Wright's Stain, without handling each slide individually as has been necessary heretofore. Many of us have attempted to devise a method by which this might be done, but preparations obtained were never satisfactory.

Since describing a modified procedure of applying Wright's Stain¹ and making a study of the variable factors in the use of this stain, we have been able to work out a procedure by which this modified Wright's Stain can be used in staining jars.

The jars used in this procedure are set up as follows:

- | | |
|-------------------------------|------------------|
| 1 Solution I ¹ | 4 Solution II |
| 2 Buffered Water ¹ | 5 Buffered Water |
| 3 Solution II ¹ | 6 Buffered Water |

In order to stain several slides at once some kind of holder is necessary. The Miller slide holder (Will Corporation, Catalog No 15672), used in many

*From the Department of Bacteriology and Pathology of Tulane University.
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laboratories, is very satisfactory. It consists simply of a number of hard rubber leaves between which the slides are placed, the slides being held securely when a thumb screw is tightened. The holder rests on the top of the staining jar, the slides hanging down into the solutions.

The solutions should come to about one-half an inch of the top of the staining jars. An ordinary straight-sided tumbler makes a satisfactory staining jar. It should be small so that the quantities of solution will not have to be too great.

The staining procedure is as follows:

- 1 Place slides in Solution I for one minute
- 2 Remove, drain off as much stain as possible, stand on end on a blotter or filter paper and *allow stain remaining to turn red*
- 3 Place in buffered water for three minutes or longer
- 4 Remove from water, drain or sling off as much as possible, and dip rapidly two or three times in Solution II (Jar 3)
- 5 Dip three or four times in Solution II (Jar 4), or allow to stand in this jar for fifteen or twenty seconds
- 6 Wash in buffered water (Jar 5)
- 7 Wash in buffered water (Jar 6)
- 8 Stand on end until dry. A staining rack which is very useful here has been described³

The buffered water should be changed frequently, as it soon becomes colored with methylene blue. Solutions I and II should be kept covered tightly when not in use. Petri dishes make fairly satisfactory covers for tumblers used as staining jars. When stains are not used constantly, it is much better to pour Solutions I and II back into stoppered bottles. Solution II from Jar 3 should not be mixed with that from Jar 4. That in Jar 3 should be discarded from time to time, the solution from Jar 4 being poured into Jar 3 and new solution placed in Jar 4.

When the alcohol has evaporated from Solution I the volume may be brought back to the original level by adding more *methyl alcohol*. Solution II, however, *cannot be made up to volume by adding ethyl alcohol* as the solution will not again become saturated for a considerable length of time after the addition of the alcohol. For this reason the level should always be brought back by *adding more of Solution II*.

Single slides are also easily stained by this method, of course, it being simpler in some ways than staining by pouring solutions on the slides. There is an added advantage of having even cleaner preparations.

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THE APPLICATION OF THE TYPHOID PHAGE PHENOMENON IN THE DIAGNOSIS OF SHIGA DYSENTERY*

By JOSEPH C. WILMETT, D.V.M., St. Louis, Mo

THE bacteriophage phenomenon is being intensely studied in its application to the treatment of infectious diseases. In certain infectious phage products have proved their usefulness as therapeutic agents.¹ The application of this principle in the Public Health Laboratory has stimulated intense work in the solving of many bacteriologic problems. It is the object of this article to discuss the use of this principle as a practical laboratory procedure in differentiating between strains of dysentery bacilli and organisms of the typhoid, paratyphoid group.

A discussion of the many difficulties encountered in differentiating the pathogenic intestinal organisms is beyond the scope of this article. It is well known, however, that occasional freshly isolated field strains of the typhoid, paratyphoid, and dysentery bacilli group do not readily yield to the various laboratory procedures of differentiation commonly used. The use of the phage principle in these cases promises to expedite diagnosis and also serve as an additional check on other differential procedures.

The phage used in this study was isolated from the stool of a typhoid termination case. Its activity was tested against a variety of organisms including dysentery bacilli, Shiga Flexner and Hiss, various stock and field cultures of *B. typhosus*, *B. paratyphosus* A and I, and *B. coli*. It proved active against Shiga Flexner and Hiss, and inactive against other organisms. However, it was decidedly more active against the Shiga strain.

Several surveys were made during the past summer in St. Louis in search of typhoid carriers. In the course of the work we encountered four patients from whom organisms, giving certain cultural characteristics of dysentery bacilli, were isolated. These freshly isolated strains were atypical in their biologic behavior, so the phage principle was used in their identification. Broth transplants of these field cultures were inoculated with anti Shiga phage and after twenty-four hours' incubation complete lysis was observed. This work was controlled with stock strains of Shiga and stock and field strains of *B. typhosus* and by omitting phage from transplants of the field culture. With further study these field cultures gave typical sugar reactions and were agglutinated by anti Shiga serum. Table I shows typical results obtained with the various test cultures after twenty-four hours' incubation.

The clinical histories in these cases did not indicate clearly whether they were chronic convalescent carriers resulting from mild attacks of dysentery or true carriers resulting from contact with active cases. None of these cases

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TABLE I
ACTION OF ANTI SHIGA PHAGE ON BROTH CULTURES*

	UNIDENTIFIED CULTURE	STOCK SHIGA	STOCK HISS Y	STOCK FLEAHER	STOCK TYPHOID	FIELD TYPHOID
Inoculated with phage	++++	++++	++	-	-	-
Not inoculated with phage	-	-	-	-	-	-

*Four plus (++++) indicates complete lysis of culture and negative (-) absence of lysis

showed clinical evidence of the disease at the time the specimens were taken. In this connection, Nichols² suggests that Shiga infections more frequently indicate chronic bacillary dysentery.

Although numerous studies of the phage principle are based upon its action against Shiga's dysentery bacilli, we find no mention of the practical use of this principle in connection with differentiation between Shiga and organisms of the typhoid and paratyphoid group for the purpose of diagnosis. It is evident, however, that it can be used in this connection only when careful consideration is given to the complexity of this principle. Extensive literature on this subject is adequately reviewed by Hadley³ so will not be discussed here.

While striving to attain the objective indicated by Bronfenbrenner⁴ who says "the hope of utilizing bacteriophage as an agent for prevention and therapy of infection lies in finding the means of removing the obstacles to its activity in its natural environment and allowing it to act freely as it does in a test tube" it is well to study the possibilities of the practical use of this principle in differentiating pathogenic bacteria for the purpose of diagnosis and in the positive identification of organisms of sanitary significance in food and water.

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A RAPID BLOOD GROUPING METHOD*

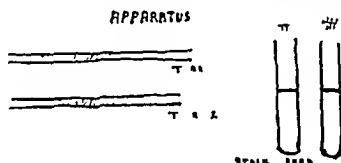
By NATHAN GROSOR, M.D., BROOKLYN, N. Y.

APPARATUS

CAPILLARY tubes, medium size, 6 inches long Capillary tubes, medium size, 5 inches long Stock sera, Group II and Group III Suspensions of recipient's and donor's red cells Sera of donor and recipient, collected in Wright's capsule tubes

PROCEDURE

The shorter capillary tube is dipped into the Group II stock serum which is drawn by capillary attraction to one half the distance. Immediately the tube is placed within the recipient's cell suspension, care being taken not to allow



air bubbles to enter between serum and cell suspension. The capillary tube is then held at either end by the thumb and index finger and inverted, allowing the cells to gravitate into the serum. This procedure is repeated with the longer capillary tube, using the type three stock serum.

Both tubes are placed under the low power lens and by proper focus one can readily note within five minutes the final result. Agglutination appears as fine cayenne pepper clumps. The eyepiece itself may be used as a direct focus upon the capillary tube.

Direct matching may be carried out in a similar manner, dipping the capillary tubes directly into the Wright capsules.

711 HOWARD AVENUE

From the Department of Pathology of the Brownsville and East New York Hospital
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ELECTROCUTION IN SACRIFICING LABORATORY ANIMALS*

BY LEO S HRDINA, CHICAGO, ILL

ELECTROCUTION deserves wider use in sacrificing laboratory animals than it has received. No means for ending life is as humane. Consciousness is instantly interrupted. At application of the current the animal stiffens and all functions cease without struggling or other agonal symptoms. Thus, no lesions are produced or chemicals introduced that may interfere with experimental findings. The method is extremely simple and applicable wherever there is 110 V alternating current, for higher voltages are entirely superfluous. (With animals larger than dogs I have had no experience.) Expense and time are minimized, and these may be factors of significance where numbers of animals are dealt with.

APPARATUS

One 10-foot length of double-ply electric light cord, fitted at one end with a standard "plug-in" fixture and at the other end with two metal spring clips as used for storage battery contacts. At the end with clips the cord is untwisted for 2 or 3 feet, to allow the clips to be separated.

METHOD

The hair on the top of the head and on the side of one thigh is wet to the skin with water, and the clips attached to the skin. If wetting is thorough, the contact is sufficient and other preparation unnecessary. The plug is then inserted in an electric outlet and left in circuit about two minutes. If on release heart action resumes, another application is made.

*From Department of Surgery, University of Chicago.
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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE M.D. ABSTRACT EDITOR

LEAD POISONING A Critical Survey of the Methods for the Determination of Lead in Biological Material Tammhill R. W. Med J Australia 1 No 16, 19, 1929

The following method is recommended as sensitive to 1 in 10 000 000

DETERMINATION OF LEAD IN URINE

Evaporation of the urine. Place 500 cc of the sample in a 7 inch porcelain basin, add 50 cc of nitric acid and evaporate on the hot plate gradually adding another 500 cc of the sample, making one liter in all. Continue the evaporation until about 30 cc are left.

Destruction of organic matter. Transfer to a silver dish (about 9 cm, 3 1/4 inches, in diameter) cleaning out the porcelain basin with 10 cc of nitric acid and finally washing it with distilled water. Allow the contents of the dish to evaporate slowly on the hot plate to avoid loss by spitting. Continue the evaporation to dryness being careful to avoid overheating which causes the mass to fume strongly and possibly to deflagrate. When dry, place the dish in a small muffle, preferably electrically heated, and heat until fuming ceases and the mass becomes charred proceeding very cautiously to prevent deflagration especially at first. Gradually increase the temperature by pushing the dish farther into the muffle until the carbon burns off quietly and the mass becomes white. Heat to between 400 and 500 C until red fumes cease to be given off usually in about twenty minutes. If the nitrates are not completely decomposed at this stage, the analysis will have to be rejected.

Separation of second group metals. Cool and add hot water until the dish is about two thirds full heat to boiling on the hot plate break up lumps with a glass rod and carefully add hydrochloric acid until the solution is clear and either neutral or very slightly acid. Then add exactly 2 cm of six times normal hydrochloric acid in excess. Special attention must be paid to the acidity of the solution at this stage. The white residue after ignition is usually alkaline and must be carefully neutralized then the required excess of acid is added. Filter off any small residue of silver through a 9 cm filter paper, wash well with hot water, catching the filtrate in a 300 cc Erlenmeyer flask. Cool and make up the bulk to 250 cc. Fit the flask with a rubber stopper with two holes through one of which a straight tube passes nearly to the bottom of the flask, the end of this tube being drawn out to a fine jet. Through the other hole is passed another glass tube bent at right angles, going through the stopper for a length of about 12 mm (half an inch). Connect up with the hydrogen sulphide apparatus and bubble a slow stream of the gas through the cold solution for one hour. Allow to stand overnight when any precipitate will settle out. Filter through a 9 cm filter paper, wash out the flask three times with a small quantity of cold hydrogen sulphide water and then wash the filter paper three times with the same liquid.

Separation of the lead as sulphate. Dissolve the precipitate of sulphides by dropping 2 cc of hot 1:1 nitric acid from a pipette around the filter paper followed by another 2 cc of the same catching the solution in the original Erlenmeyer flask. Wash six times with hot distilled water. Warm the solution in the flask and evaporate to about 20 cc, then transfer to 50 cc cylindrical beaker (nonalkali glass) washing out the flask 3 times with warm water. Evaporate quietly to about 5 cc on the hot plate then add 1 cc of sulphuric acid and heat until strong fumes arise. Cool and add 20 cc of cold water then 10 cc of alcohol mix well and allow to stand overnight. Filter through a 9 cm filter paper washing out the beaker with a solution of 60 volumes water 32 volumes of absolute alcohol and 3 volumes of sulphuric acid. Then wash the filter paper 3 times with the same solution.

Colorimetric determination of the lead Dissolve any precipitate off the filter paper by dropping 5 cc of hot (1 2) ammonium acetate solution from a pipette around the filter paper, followed by another 5 cc of the same reagent, catching the solution in the original 50 cc beaker Wash 6 times with hot water Cool and Nesslerize, using a standard solution of lead acetate of 0.0001 gm of lead in 1 cc

The Nesslerizing is carried out in the following manner Transfer the assay solution to a 50 cc Nessler tube, add 1 cc of a 10 per cent potassium cyanide solution, 1 cc of ammonium hydroxide and 6 drops of freshly prepared ammonium sulphide Make up to the 50 cc mark and mix well This assay solution must be quite colorless Add to it the standard lead acetate solution, stirring after each addition until the color matches that of the assay, when both tubes are placed in a colorimeter It is advisable to arrange the strength of the assay solution so that not more than 8 cc of the standard lead solution will be required for Nesslerizing If much lead sulphate precipitate is noted after the addition of water and alcohol, the solution in ammonium acetate must be made up to a definite volume and an aliquot part taken for Nesslerizing, so that not more than 8 cc of standard solution will be used in the comparison In this case use the same proportion of (1 2) ammonium acetate for making up the comparison solution as is contained in the aliquot part of the assay solution It is desirable in all cases to regard the first comparison as a trial and to make up another standard in which the required lead acetate solution is added before the ammonium sulphide and along with the ammonium acetate, potassium cyanide, ammonia and water to make up a total of 50 cc The solution is mixed well and 0.3 cc of ammonium sulphide added and after further mixing compared with the assay Slight additions may then be made to either assay or standard in order to match them and the necessary corrections made

THE CHROMATE METHOD FOR THE DETERMINATION OF LEAD

Ashing Before analysis, tissues and feces must be freed from water by baking This may be done very rapidly by heating the material in porcelain dishes on a hot plate until it starts to char Transfer to the muffle furnace and ash to a dull red heat Fecal matter usually ashes readily, but the tissues form a residue which must be repeatedly extracted before the entire char is consumed Usually most material requires reashing as a certain quantity of inorganic salts becomes fused and prevents complete oxidation After the first ashing, the material should be cooled and extracted with dilute hydrochloric acid and hot water It is essential that at this stage all the ash be dissolved, for frequently lead phosphate is present as an insoluble residue that might be mistaken for silica If this residue is insoluble in hydrochloric acid, it should be treated with a mixture of hydrochloric acid and tartaric acids (which dissolves lead phosphate) until the ash is quantitatively dissolved

In analyzing urine for lead, the procedure has been to evaporate to dryness, char and ash the residue Since urine residues are difficult to ash, because of the large quantity of inorganic salts present, repeated extraction and ashing is necessary if all the salts are to be dissolved This makes the process burdensome The following new method in which evaporation is avoided by precipitating lead directly from the urine, has been devised

Entrainment Ammonium hydroxide is added to urine until it is strongly ammoniacal The mixture is allowed to stand from one to twenty four hours In this reaction the earthy phosphates are precipitated and lead phosphate is carried down quantitatively by entrainment The gelatinous mass of phosphates settles into a compact mass from which the clear lead free liquor can be decanted and the remainder filtered by suction on a Buchner funnel The filter paper containing the precipitate, ashes readily in a few minutes and the quantity of lead may be determined by the chromate method as follows

Precipitation The strongly acid solution should be neutralized with sodium hydroxide Hydrochloric acid should be added until the solution is just acid to methyl orange Saturate the cold solution with hydrogen sulphide, if sulphides precipitate to any great extent during the process, they may be filtered at once, but if no precipitate appears, the solution saturated with hydrogen sulphide should be allowed to stand overnight before filtration Immediately after filtration the precipitate should be washed as lead sulphide oxidizes rapidly when in

contact with air. Solution of the washed precipitate in nitric acid, boiling to expel hydrogen sulphide, cooling and finally neutralizing with sodium hydroxide, as indicated by phenol phthalein, are then necessary. After reacidification with acetic acid, two or three drops of a saturated solution of potassium chromate should be added. To hasten the reaction the solution should be boiled for a few minutes. If no turbidity is present, the solution should stand overnight before filtration to allow separation of the extremely small amount of lead it may contain. After filtering all trace of soluble chromate should be washed from the filter paper.

Titration. The chromate is dissolved in dilute hydrochloric acid, an excess of potassium iodide added at once and the free iodine titrated with 0.005 normal thiosulphate solution, starch being used as an indicator.

ACETONE IN BLOOD AND URINE A Method for the Detection of Acetone Wall hanser A. J A M A 91 31, 1928

Reagent (Scott Wilson)

"Ten grams of mercuric cyanide is dissolved in 600 cc of water. Add a cooled solution of 180 gm of sodium hydroxide in 600 cc of water. Transfer this mixture to a heavy glass jar and add 20 gm of silver nitrate dissolved in 400 cc of water. This must be added in a fine slow stream with vigorous stirring constantly. The silver dissolves completely giving a clear solution. If it is turbid it may be set aside and the clear supernatant fluid removed by a siphon after three or four days. This solution will keep for six months after which a new solution must be made. Keep in a tightly stoppered dark bottle." The stopper of the container may conveniently carry a pipette graduated in twentieths of a cubic centimeter. A rubber bulb should not be used on the pipette.

Method. The urine or blood is placed in a suitable container. The original container used in most hospitals for urine specimens is generally employed, no attempt being made to measure the volume. One drop of Scott Wilson reagent is placed on a microscopic slide which is then inverted to form a hanging drop. This is placed over the mouth of the container, care being taken that the reagent does not come in contact with the neck of the bottle, in order that precipitation may not occur through direct contact with the urine. Any time of exposure may be made but an exposure of two minutes has been established as this period has been found to give the best quantitative results. After the time period has elapsed the hanging drop is inspected for the microscopic appearance of a fine white clouding or precipitate. If there is such a precipitate or clouding the test is positive for acetone. If the reagent remains unchanged, acetone is negative.

ACHYLIA GASTRICA The Excretion of Neutral Red in the Stomach Winkelstein A and Marcus J M J A M A 92 No 15 1238, 1929

The following modified technic is described

The patients presented themselves with fasting stomachs, the Rebuffs tube was passed and 40 mg of neutral red dissolved in a few cubic centimeters of sterile distilled water was injected intramuscularly (gluteal or deltoid). The patients were then given at once, 250 cc of strained oatmeal gruel. A sample was aspirated every fifteen minutes for two hours. This procedure was carried out in 60 cases of achylia gastrica. All of these patients had had an Ewald and at least one fractional test meal and often two or three with bouillon, alcohol or oatmeal gruel. In 15 cases, histamine (Imido Roche 7 1/4 minims, 0.45 cc, subcutaneously) was administered in addition to the neutral red and the test meal. Neither the dye nor free hydrochloric acid appeared in the gastric contents. With reference to certain criticisms one should state that bile stained reddish brown by the dye can readily be excluded by the practiced eye as a source of error. A consideration of the results of this study shows that, irrespective of the disease present the dyestuff is not excreted for two hours when a true achlorhydria is present. In the group are included 6 cases of pernicious anemia, 10 of gastric carcinoma, 3 of cholelithiasis, 17 patients with subtotal gastrectomy for ulcer, and 2 proved cases of gastric syphilis. It is obvious that true achylia gastrica,

or, better expressed, true achlorhydria, occurs in a variety of conditions and that the failure of the neutral red to appear is linked up with the absence of the acid and is not determined by the disease present

The following conclusions are advanced

- 1 Neutral red is excreted into the stomach whenever free hydrochloric acid is secreted
- 2 Neutral red is invariably excreted in the false achlorhydrias
- 3 It is not excreted in true achylia gastrica, whatever the cause of the achylia may be
- 4 Neutral red gives as much information as histamine and is preferable to the latter for routine use
- 5 Neutral red is helpful in the study of the normal and pathologic physiology of gastric secretion and in the differential diagnosis of the diseases producing false and true achylia gastrica
- 6 The use of neutral red in every case in which achylia gastrica is suspected is advocated

GONORRHEA The Oxidase Reaction in the Laboratory Diagnosis of, Price, I N O
British M J, 199, February 2, 1929

After microscopic examination of suspicious forty eight hour colonies, if the organisms resemble gonococci morphologically the surface of the medium is gently washed with about 0.5 c.c. of 1 per cent solution of dimethyl paraphenylene diamine hydrochloride and examined after one, three, five, ten, fifteen, twenty, and thirty minutes for any color change in the colony under observation

A positive reaction is shown by the appearance (in gonococcus) of a gradually deepening pink color, changed to red, then to reddish purple, and finally in thirty minutes to jet black

The same reaction is given by M. catarrhalis but appears more rapidly

GONOCOCCUS Successful Cultivation on Blood Agar Plates, Herrold, R D J Infect
Dis 42 No 1, 79, 1928

Formula

- 10 gm peptone Witte
- 7.5 to 10 gm bacteragar
- 1 gm dibasic potassium phosphate
- 50 gm bactobeef dehydrated

The bactobeef is added to one liter of water and after standing overnight the juice is squeezed out and made up to one liter with distilled water. The reaction is adjusted to pH 7.4 to 7.6

To the melted agar at 65° C add 10 to 15 per cent of defibrinated sheep blood or whole human blood, cool to 45° C and pour as plates or slants

In the author's experience this medium is very successful for primary cultures

FLAGELLA A Method of Staining Bacterial Flagella, Craigie, J Brit J Exper Path
9 No 2, 55, 1928

This is essentially a modification of Zettnow's method

The usual precautions as to clean glassware are obligatory

Use twenty four hour cultures on agar with plenty of water of condensation and pipette off the growth in the condensation water, which is at once dropped into "fixative solution". The fixative solution consists of saline containing 2 per cent of formalin. Formalized phosphate buffer solution of pH may be used instead of saline. Leave in fixative solution for one hour or, better, overnight, before attempting to stain. These formalized suspensions keep for months at room temperature

Preparation of Films In a clean tube put distilled water and add thereto the formalized suspension until faint opacity appears. Spread gently a small loopful of this on

a slide cleaned as above. Dry at 37° C the slide being inclined so that varying degrees of thickness of suspension are obtained. Then heat at 90° to 100° C for five minutes or longer (This can be done conveniently on the lid of a small steam sterilizer such as is used for sterilizing pipettes or syringes.)

Place in jar of distilled water for five minutes and wash with distilled water. Dry and heat again at 90° to 100° C when preparations may be mordanted.

Mordanting. Dissolve 10 gm of tannic acid (this must be light and pure, not of commercial quality) in 200 cc of distilled water, heat to about 60° C and slowly add with frequent agitation 30 cc of 5 per cent aqueous solution of tartar emetic. A crystal of thymol is necessary if the mordant is to be stored.

The slide is flooded with mordant and heated for five to ten minutes at 90° to 100° C. When doing this the slides should be under constant observation, and when any tendency to drying of the mordant is observed at the edges of the slides more mordant should be run on and guided along the dry edge with a small glass rod. Remove slide and flood off mordant under tap. Any dry mordant at the edges of the slides which is easily seen, as it becomes white on cooling must be removed with a clean cloth. Wash in distilled water.

Silvering. Dissolve 1 gm silver sulphate (B D H brand used) in 200 cc of distilled water and store in light tight bottle.

To 20 cc of this silver sulphate solution add mono ethylamine solution (33 per cent B D H) until the resulting opresity just clears up. More silver may be added if the ethylamine has been added in excess. About 4 to 5 drops of ethylamine are required for 20 cc of silver solution. Flood slides with silver ethylamine mixture thus prepared, and warm over tip of luminous flame until preparation just begins to steam. Allow to continue steaming, but do not overheat, until the preparation turns brown or black according to degree of reduction. It is emphasized that to obtain the best preparations heating should not be continued after the preparation has turned brown. If a black metallic cloud appears in the solution, wash off and replace with fresh silver ethylamine. When sufficiently reduced flood off under tap care being so taken as to flood the slide that no metallic film settles on the preparation. Wash in distilled water. (If desired one can mount the preparation after drying, but such preparations fade rapidly especially if the silver has not been completely reduced, and it has already been noted that complete reduction does not give optimum preparations.)

Gold "toning" Prepare toning solution by adding 20 drops of 1 per cent solution of gold chloride to 20 cc of distilled water. The gold solution was made from ordinary photographic gold chloride. Immerse slides in this weak solution of gold chloride and expose to daylight for thirty minutes or longer. Wash and dry.

When greater density and contrast is desired the following alternative to gold "toning" may be adopted with advantage.

Immerse slide for five minutes or longer in a 0.1 per cent solution of uranium chloride. Wash and apply the following developer for one to two minutes.

Pyrogallie acid (Merck), 0.5 per cent aqueous solution, 10 cc

Liquor ammoniac fort, 3 to 5 drops

Mix and use immediately

Wash slide and dry

This developer may be applied also to the gold preparation.

Mounting preparations. Preparations are mounted in balsam without solvent, the balsam being rendered fluid by heat. This method gives very delicate permanent preparations.

OLIGODENDROGLIA. Method of Staining Oligodendroglia and Microglia, Penfield W Am J Path 4 No 2 153, 1923

1 **Harden.** Tissue in 10 per cent formalin (or formalin ammonium bromide) for an indefinite period. About a week in formalin gives excellent results.

2 **Section.** Cut sections at 20 microns on the freezing microtome and receive them in 1 per cent formalin or distilled water. Through the succeeding steps the sections should be handled by a glass rod shaped like a hockey stick.

3 Deformalinize Place sections in dish of distilled water to which 10 to 15 drops of strong ammonia have been added and cover so as to prevent escape of ammonia. Leave in this solution overnight to remove formalin

4 Bromate Transfer sections directly to Globus' hydrobromic acid in 5 per cent solution (5 cc of 40 per cent hydrobromic acid plus 95 cc distilled water). Place in incubator at 38° C for one hour

5 Wash Pass through three changes of water (a, b, c)

6 Mordant Place sections in 5 per cent solution of sodium carbonate for one hour (Sections may remain here five to six hours without ill effect)

7 Impregnate Pass sections with or without washing direct to Del Rio Hortega's silver carbonate, weak solution,* and leave them here three to five minutes. Sometimes they may be left until they begin to turn a yellowish gray. Then transfer them to the reducer. Control the duration in silver solution by taking out a section at intervals of one to two minutes and examining under the microscope. The sections should turn a smooth gray color in the following reducer

8 Reduce Plunge into 1 per cent formalin and agitate

9 Wash Distilled water

10 Tone Leave in gold chloride (1:500) at room temperature until all yellow tint disappears and the sections are a smooth bluish gray

11 Fix Hyposulphite of soda (5 per cent photographic "hypo")

12 Wash Distilled water

13 Dehydration may be done conveniently after Del Rio Hortega's custom as follows. Float sections on the slide and flatten out with needle. Wash with two to four changes of 95 per cent alcohol from a drop bottle. Follow this with a few drops of carbol xylol creosote (proportion of 1:15). When clear, drain slide and blot immediately with two thicknesses of fine filter paper. Mount in Canada balsam

ACIDOPHILUS MILK Preparation of, Rice, F E Am J Pub Health 9 1105, 1928

Carefully clean a quart thermos bottle by allowing it to stand overnight full of water containing some washing powder or a little household ammonia, then discard it. Place the cork, a can opener and thermometer in a pan and pour boiling water over them. Wipe the top of a one pound can of evaporated milk free from dust and pour boiling water over it. Open the can and pour contents into the pan that has been scalded. Fill the can with boiling water and pour into the evaporated milk. Immerse the pan in cool water and stir the mixture with the thermometer until the temperature comes down to about 105° F. Add 2 or 3 ounces of commercial B acidophilus culture, mix, and transfer to the thermos bottle (The temperature should now be between 100° and 102° F). Cork and let stand for twenty four hours, or until the milk has acquired a pleasantly sour taste. When this is attained, transfer to a clean milk bottle and place in the refrigerator.

Succeeding cultures of acidophilus are prepared by using about a tereupful (6 ounces) of milk culture previously made to inoculate the diluted evaporated milk for the next run. Proceeding in such a manner it will be found that acid is produced at a more rapid rate than when the first quart was prepared using the commercial broth culture as a starter. Thirteen to seventeen hours are now quite sufficient. If fermentation is allowed to proceed for a longer time so much acid is developed that the taste becomes unpleasantly sour.

After a little experience one may stop the action of the bacteria at any desired degree of sourness. This is effected by merely transferring the milk to a clean glass bottle and placing in the refrigerator. It is perfectly safe to keep the culture at room temperature, but,

*Silver nitrate (Merck) 10 per cent solution----- 5 cc
Sodium carbonate (pure) 5 per cent solution----- 20 cc
Ammonium hydroxide (sufficient to dissolve the precipitate)
Distilled water ad ----- 75 cc

The ammonium hydroxide in strong fresh solution should be added drop by drop until the precipitate is just dissolved stirring the solution all the while. It is important not to add too much ammonia. A fine black sediment may remain behind which does not resemble the more voluminous precipitate of silver carbonate. This fine sediment should be filtered off. The solution may then be preserved in a dark bottle for long periods.

as has already been mentioned, a considerable increase in acidity may be expected. This is of little consequence, however, if the product is consumed within twenty four hours. On the other hand, if the milk is kept in a refrigerator it should be consumed within forty eight hours for the reason that the organisms rapidly die out at such low temperatures.

SPIROCHETES Some New Spirochete Stains Gulstein M and Dhar D B Dermnt Wehnschr 86 45, 1928

1 Ferric (Chrom) sulphate tannin methyl violet stain. A concentrated solution of ferric sulphate, chrom sulphate or chrom alum 30 per cent tannin solution 1 per cent watery methyl violet. Treponema is suspended in two or three loops of first solution and rubbed into it on the microscopic slide. A few minutes later specimens are prepared by wiping the mixture on slides. They are dried and fixed by heating. Then they are soaked in distilled water, and tannin solution poured over them. It is left for five minutes or longer, rinsed, and the methyl violet left on for three or five minutes.

2 Carbolfuchsin tannin potassium antimonyl tartrate methylene blue stain. Aside from Ziehl's solution 5 per cent tannin 10 per cent solution of potassium antimonyl tartrate and 1 per cent watery solution of methylene blue. After the smears have been fixed with alcohol or by heat the oral spirochetes are stained with Ziehl's solution, boiling for two to five minutes, rinsed and treated with tannin solution. After second rinsing exposed to the potassium solution for five minutes, renewed rinsing and exposed for from one to three minutes to methylene blue. If methylene stain is continued too long spirochetes also take the blue color. Treponema stain held light red, while bacteria and cocci have a red body and blue ectoplasm.

3 Supravital stain with Victoria dyes. A loop of buccal spirochetes is rubbed on a slide with a drop of physiologic saline. Then two or three loops of a 2 to 5 per 1000 solution of Victoria blue are added, well mixed and a cover glass is applied.

NEUROGLIA Staining Fibrillary Neuroglia in Formalin Fixed Material, Davidoff L M Am J Path 4 No 5 493, 1928

1 Blocks of tissue 2 or 3 mm thick are cut from the formalin fixed brain, cord, or tumor, and placed in a dish filled with about 100 cc of distilled water containing 30 to 40 drops of strong ammonia water. This is kept air tight in an oven at 30 C for four days.

2 The blocks are washed for twelve to twenty four hours in running water.

3 They are then fixed in Zenker's solution for twenty four hours.

4 Embed, cut, and stain.

The Stain

1 Mixture of methyl violet 1.0 gm } accurately weighed
orange G 0.5

2 Add 100 cc distilled water and stir thoroughly.

3 Place in or on a warm oven (37 C) for twelve to twenty four hours to precipitate.

4 Decant supernatant fluid and wash precipitate several times with distilled water.

5 Place in oven to dry.

6 Make a saturated solution of dried precipitate in absolute alcohol.

This solution if well stoppered, will keep indefinitely.

For staining use one part stock solution to three parts of 20 per cent alcohol.

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building,
Richmond, Va

*Diseases of the Stomach**

IN THE production of this volume Dr Reh fuss has made a great contribution to medicine. It contains a veritable mine of valuable information for practitioner and gastroenterologist alike. Written with an ease of style possible only for those who have attained mastery of their subject, it becomes a piece of medical literature.

The volume is rather comprehensive for textbook purposes. It should probably find its greatest value as a book of reference for practitioners and students. On the other hand, the gastroenterologist's library is incomplete without it.

The author is to be commended upon the way in which he presents his subject matter. His approach to the main theme is logical and sequential. The work is divided into three parts as follows. Part I contains chapters on anatomy, embryology, physiology, laboratory methods, x ray, and gastroscopy. Part II deals with the diseases of the stomach and Part III is for the most part taken up with a consideration of the effect of gastric diseases upon other important organs. The work abounds in helpful illustrations, charts, diet lists, and regimens.

Principles of Pathology†

THE task confronting the writer of textbooks is becoming increasingly difficult. He must determine, first, to which audience his effort shall be addressed, the student, the practitioner, or the specialist, and, second, whether he shall endeavor to present all the minutiae of the subject, or only those phases of it which may be described with certitude.

And, in the background, there is always the possibility that much of what he has written laboriously and after painstaking labor, may become obsolete through the advances made in the subject.

Power and Hala have selected the student and the practitioner as their audience and have written their book both for those who approach the subject for the first time or who desire to refresh their memories "on points long forgotten, or in regard to recent discoveries."

They have endeavored, therefore, to prepare a concise text in language not overburdened with exuberant nomenclature and understandable to the novice.

In this undertaking they have been eminently successful. As said in the preface "There is very little that cannot be said in plain English and that is how we prefer to say it."

Very wisely, there is no attempt to make the book encyclopedic, the authors concentrating rather on what is essential and important leaving the rare and unusual to monographs.

The book may be warmly recommended not only to the student and practitioner but to all who desire a succinct presentation of the basic principles of pathology sometimes overlooked when "the forest cannot be seen for the trees."

**Diagnosis and Treatment of Diseases of the Stomach with an Introduction to Practical Gastro-Enterology*. By Martin E. Reh fuss, M.D., Assistant Professor of Medicine in Jefferson Medical College. Reprinted one month after publication. Octavo of 1236 pages with 519 illustrations some in colors. Cloth. W. B. Saunders Company Philadelphia, Pa.

†*Principles of Pathology for Practitioners and Students*. By H. d. Arcy Power, Professor of Pathology, College of Physicians and Surgeons, San Francisco and William W. Hala, Assistant Professor of Pathology, Long Island College Hospital, Brooklyn. Cloth. 787 pages. 298 illustrations. 12 in color.

NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

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EDITORIALS

The Bacteriology of Genitourinary Tract Infections

NONSPECIFIC infections of the genitourinary tract in the vast majority of cases are usually regarded as due to *B. coli* if pus cells and gram negative bacilli of conformable morphology are found in the urine. It is seldom that extensive bacteriologic studies are carried out in confirmation of the diagnosis and still less frequent are reports of studies of the bacterial flora of the genitourinary tract, although the presence of adventitious bacteria is often a complicating factor in the determination of the etiology of a pyelitis, for example.

Recent studies along this line, therefore, are of some interest and possess, perhaps, some clinical application warranting a survey of the data so far obtained.

A study of two hundred cultures of gram negative bacilli isolated from two hundred cases of genitourinary infection has recently been presented by

Hill, Seidman, Stadnichenko, and Ellis¹ representing an endeavor to determine the bacteriologic characteristics of the organisms and their possible clinical correlation

The study is complicated not only by the constant presence of adventitious organisms but also by the multiplicity of bacteria somewhat loosely classified as belonging to the colon group

To avoid the former difficulty, before the collection of the specimen, these workers first cleansed the urinary meatus with alcohol and the anterior urethra by an injection of 1 500 meroxyol

The second factor necessitated an extensive and varied bacteriologic study, the results of which form the subject of the report

These workers are convinced, first of all, that "there must be a careful correlation of thorough examinations of direct smears with cultural results if accurate bacteriologic findings are to be obtained "

Rarely, in cases undergoing intensive treatment, organisms may be absent in the smear and recovered by culture but, in general, "an organism which develops scantily on culture, usually only after forty-eight hours of incubation, and which has not been seen in the direct smear, can be discarded as a urethral contamination "

The cultures studied in this series are divided for convenience into four groups

I One hundred *Escherichia* cultures, fermenting lactose with acid and gas, not producing acetyl-methyl-carbinol, but methyl-red positive

II Seventy-nine *Aerobacter* cultures, fermenting lactose with acid and gas, producing acetyl-methyl-carbinol, but methyl-red negative

III Five *Proteus* cultures

IV Sixteen miscellaneous cultures

Attention is called to the differential value of citrate media which the *Aerobacter* forms utilize promptly and the *Escherichia* belatedly, scantily, or not at all

The most significant facts derived from the correlation of bacterial groups with the different types of clinical infections encountered are thus summarized

1 Seventy-five per cent of the blood stream invasions were due to organisms of Group II (*Aerobacter*) in the 12 cases in which the same organism was recovered from the blood and urine the incidence of Group I (*Escherichia*) being only 1, or 8.3 per cent This high proportion of *Aerobacter* cultures in such blood stream infections parallels the high incidence of this group of cultures in genitourinary infections as compared with organisms present in the intestinal flora

This observation suggesting a differentiation of the colon group in blood stream invasions is, apparently, new and suggests the necessity of further studies along this line

2 In 25 cases of lithiasis, Group II (*Aerobacter*) organisms were encountered in 48 per cent, and *proteus* in 12 per cent, the remainder (40 per cent) being Group I (*Escherichia*)

3 In three cases of abscess formation, two developed blood stream invasions

In a further study Stadnichenko reports upon thirty strains of gram positive cocci isolated from genitourinary infections, the most prevalent type of infection being prostatitis, although there was no correlation between the type of infection and the organisms isolated

In fifty cases bacteriologically studied Gondolf and Stringer³ found that over half the cases proved to be organisms other than the Escherichia group

The results thus summarized cast grave doubt upon the efficiency of the stock *B. coli* vaccines not infrequently resorted to in the treatment of this type of infection

Finally, Scudder and Belding⁴ report upon the characteristics of a group of higher bacteria from the genitourinary tract belonging apparently, among what used to be classified as cladothrix or streptothrix, which may be mistaken for streptococci, and which have, perhaps some relation to chronic infections in the urinary tract

It would appear that this subject is worthy of extensive and, of necessity prolonged study

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—R A K

What Is an Internist?

THE term, Internal Medicine has come within recent years into almost universal usage And yet few terms appear to be more difficult to define A medical organization of national prominence has in the past had as a part of its program the education of the lay public in the significance of this term The directory of The American Medical Association classifies internist or internal medicine as a separate and distinct specialty And yet how many can formulate a concise definition of the term

The present writer having made the attempt and failed, undertook to obtain a series of definitions or expressions of opinion from a group of pre eminent so called internists throughout the country Questionnaires were also sent to a few men in specialties other than internal medicine The results are sufficiently interesting to bear analysis

Those replying may be classified roughly into three groups first, men whose primary interest is the teaching of medicine in our leading medical schools, second, those primarily interested in the practice of internal medicine The viewpoint of this second group might be expected to differ slightly from the first inasmuch as members of the second have, theoretically at least,

had more contact with borderline cases, diseases that might be treated in any one of several specialties such as dermatology, neurology, and pediatrics. The third classification includes men practicing these other specialties who might be expected to voice the criticism that the internist trespasses upon their field of work.

Twenty-three definitions or descriptions are at hand. As might have been anticipated, the classifications of the replies bear no relationship to the three groups mentioned above. Nearly all agree on the great difficulty of defining an internist or internal medicine and many prefer to submit a description rather than a definition.

GROUP I

Three offered interpretations based on the literal significance of the term, relating it to diseases of the internal organs. They are as follows:

1 "Until I received your letter I had never tried to define internist or internal medicine. Had anyone asked me casually what was meant by these words I should have given him at once a definition. Now when I try to put it in writing I find the thing is not as easy as I thought. However, I should like to submit the following:

"An internist concerns himself with the diagnosis and treatment of diseases having their seat in internal structures and tissues of the body. He excludes from his field the diseases peculiar to the organs of special sense, the diseases of infancy, and those methods of treatment that belong to the category of surgical operations.

"Internal medicine is a branch of medicine that concerns itself with the diagnosis of diseases of the internal organs and tissues of the body, and with their treatment in so far as such treatment does not involve the use of surgical procedures and methods that belong to the well defined specialties of medicine."

2 "I notice that the Dorland's American Illustrated Dictionary defines an internist as a physician who treats diseases of the internal organs. Thus, I think, is not a very good definition unless one qualifies it by saying exclusive of the organs of generation. If this qualification is not made then the internist becomes an obstetrician and gynecologist. It may be added that such a definition is not sufficiently inclusive because an internist also treats peripheral neuritis, vascular diseases of the limbs, etc., which are certainly not internal organs. The reason why it is so difficult to write a concise definition is that the work of an internist is not limited by anatomical or physiologic considerations but by custom. I doubt whether any definition of an internist can be formulated briefly. Of course one might go further and say that an internist should not treat psychiatric or neurologic conditions. The more I think of it, it is impossible to draw any hard and fast line as to just what an internist should cover."

3 "I have delayed answering your letter about a definition of the expression, 'internal medicine' and 'internist,' because I have found this so hard to do. How would this do?

"Internal medicine is concerned with everything which influences the general health or nervous stability of the patient. It is particularly concerned with disorders of the organs embraced in the thorax and the abdominal cavity."

It is impossible in the above group as well as in those that will follow to limit any one reply to a single group, for many of them include features of two or more of these classifications.

GROUP II

The next group roughly comprises those who, instead of *defining* internal medicine and an internist, *describe* them, chiefly in terms of the limitations of the field with respect to the other specialties.

4 "Internal medicine is a branch of the practice of medicine that has to do with the diagnosis of medical and surgical diseases and with the treatment of medical diseases which are not entirely within the scope of the more highly specialized branches of medicine such as dermatology, neurology and pediatrics."

5 "I should define an internist as one who concerns himself with the diagnosis and the nonsurgical treatment of diseases involving any organ or tissue in the adolescent or adult human body except the skin, the nose and throat and the special senses."

"Internal medicine is concerned with the same subjects. Obstetrics is excluded because childbirth is not a disease. The toxic diseased conditions associated with it however (eclampsia, anemia, vomiting) are within internal medicine."

6 "I think it is very hard to define internal medicine or an internist. Either can be described better than defined. I have always looked on internal medicine as being that part of medicine which was left over after operative surgery, obstetrics and the specialties which require technical procedures in diagnosis or treatment have been taken away. This is certainly not a definition and probably a poor description."

7 "Internal medicine is medicine minus surgery and the specialties."

"An internist is one who devotes himself intensively to internal medicine and thus becomes a specialist in internal medicine."

8 "By an internist I mean a man who practices internal medicine as distinguished from the various specialties which he should of course leave severely alone."

"The two fields on which he could encroach to some extent would be pediatrics and neurology. As an internist I feel at perfect liberty to take care of children over two years of age either at my office or at their homes. I do think that infants are out of our field. Neurology has always been closely allied to internal medicine and is included in all the textbooks on the subject. My rule here would be to call on a neurologist when I was up a tree."

"The various subdivisions of internal medicine are now becoming specialties but that does not preclude their being handled by the internist. I refer of course to cardiology, gastroenterology, metabolism, tuberculosis, syphilis and the diseases of allergy. I use specialists on these subjects for convenience. In other words if I am perplexed over a case I refer it to one of them for diagnosis and possible treatment. As long as I feel equal to the situation I keep the patient under my own care."

"I do not believe a real internist should ever touch a scalpel nor do I believe that he should handle conditions in the nose or throat or genitourinary tract."

"Some of the skin diseases are so dependent on internal medicine that they could better be handled by the internist than by the dermatologist. I refer particularly to the allergic conditions."

One correspondent whose definition appears under Group IV offered the following remarks relative to this method of defining internal medicine by describing its limitations:

9 "It seems to me that the so-called medical specialties inclusive of neurology, gastroenterology, cardiology, hematology, pediatrics and genitourinary medicine (aside from surgical therapy) all belong in internal medicine."

"Obstetrics is of course excluded from internal medicine."

"The suggestion that internal medicine is distinguished from the various specialties and that the internist should leave the specialties severely alone could not in my opinion be adopted, for if it were the internist would have nothing to do since practically every part of internal medicine is included in one or another specialty."

"Instruments of precision are used by internists as well as by all specialists in medicine and in surgery for diagnostic purposes. The ophthalmoscope for example is a necessary instrument in the work of the internist."

"Moreover, laboratory methods must be applied by all medical practitioners, not surgeons, and specialists."

"The internist must also be able to make diagnoses in diseases that require surgical treatment, for example, appendicitis, cholecystitis, Banti's disease, nephrolithiasis, brain tumor, etc "

GROUP III

Roughly the replies in this group express the opinion that internal medicine cannot be closely defined as a specialty but includes a very broad territory, taking in portions of many of the specialties and yet at the same time being distinct from the field covered by the so-called general practitioner. There is no attempt at rigid delineation of the internist's field of activities.

10 "I think that Dr Osler was one of the first in this country, possibly the first, to employ the term, internal medicine. I quote from the first paragraph of his essay on Internal Medicine as a Vocation, published in the volume entitled *Aequanimitas*. He says

"I wish there were another term to designate the wide field of medical practice which remains after the separation of surgery, midwifery, and gynecology. Not itself a specialty (though it embraces at least half a dozen), its cultivators cannot be called specialists, but bear without reproach the good old name physician, in contradistinction to general practitioners, surgeons, obstetricians, and gynecologists."

"The term originated in Germany where it is used synonymously with clinical medicine. I have always employed the term in the sense in which it was used by Dr Osler."

11 "I certainly do not differ from you in finding it difficult to define an internist. Of course he is one who is concerned only with nonsurgical diseases, and it has been my understanding that the term has been derived from the fact that most of the diseases which come to the hands of the internist are of the internal viscera, and to employ these of broader significance, usually systemic in character. I should say that the term expressed exactly the opposite of the term general surgeon."

12 "According to my understanding *internist* is nearly synonymous with *physician*. General practitioner is more inclusive, for he, like the country doctor, will usually undertake to do at least minor surgery. As a matter of fact the old term *physician* seems to me to have especially connoted doctors whose chief employment was the treatment of disease by any methods, including those of minor surgery, he was a 'general practitioner'.

"The term *internist* seems to have come into vogue since the development of *laboratory medicine* as a distinct specialty.

"Laboratory methods have tended to exalt *diagnosis* and to more or less segregate it as a specialty based on exact explorations.

"A new branch of medical science and art is represented in the technique, and its interpretative judgment, developed in the clinical laboratory.

"Physicians trained to appreciate this new source of information naturally turn to it constantly, not only for suggestive and confirmatory diagnosis but for crucial evidence of the effects of *treatment*.

"With this increase of exactness in the appreciation of pathologic complexes the physician has naturally more and more eschewed the use of things and methods with which he is not intimately conversant. He seeks to be a specialist in any task he undertakes, he therefore lops off surgery from his practice in toto. He confines himself to that systematization of phenomenon which we know as diagnosis, and he selects therefrom for experimental study (treatment), such complexes as have for him particular interest.

"He is, I take it, an *internist*. Woe is you! Boil this down to a sentence."

13 "It has always seemed to me that internal medicine was a very incorrect way of designating that branch of medicine which includes everything not surgical. So, too, the designation of the man practicing nonsurgical medicine as an internist.

"The more one thinks of the type of work the nonsurgical practicing physician does in his daily routine, the more difficult becomes a concrete, concise definition thereof.

"The nonsurgical men in their diagnostic work, if they are up to the minute in the progress of their profession, are called upon to do and try to accomplish diagnosis in nearly the entire field of medicine even invading the ophthalmologic area. Nevertheless in their clinical work or in the field of therapeutics they studiously avoid all of those disorders which call for the application of surgical methods."

14 "The question embodied in your letter is certainly a fairer. After giving the question due consideration, here goes my definition:

"Internal medicine concerns itself with the clinical pathology of the so-called general diseases but should include a thorough grounding in the specialties."

"An internist is one who concerns himself with the diagnosis and treatment of general medical conditions but to profess such he should be thoroughly grounded in all the special fields of medicine."

15 "My ideas regarding specialization in medicine are quite old-fashioned. I believe that every medical man should have a thorough general medical education and that he should practice general medicine for some time before he ventures upon a specialty. This I believe to be as important in the case of internists as in the case of a neurologist or psychiatrist."

"I cannot give you an accurate definition of an internist but he is or should represent in his activities general medicine. He should be a man who is familiar not only with visceral diseases and the general affections to which the organism is subject, but also should have a reasonable knowledge of diseases of the eye, ear, skin and of the brain and spinal cord besides an acquaintance with the problems of psychiatry. While he cannot of course be a 'universal specialist' these qualities which I have mentioned are in my judgment eminently desirable."

16 "Though internal medicine is extensively used, it is interesting that so far as I know no chair in any medical school in the United States carries the defining adjective 'internal' but instead 'medicine' is used with in some of the old medical schools the use of the term 'physic' instead of 'medicine'. I have an idea that we would have done best if we had held to this terminology and used medicine rather than internal medicine."

GROUP IV

In this group the idea is emphasized that an internist by virtue of special training and experience has developed a broader perspective and is therefore capable of clearer insight than his fellow practitioner. The first two contributions in this group are perhaps two of the most successful attempts at a brief, concise definition. More exception could be found for the first than for the second. Here as elsewhere throughout these classifications definitions which are included in other groups might be included in part at least within this group.

17 "Internal medicine is the art and science of correctly interpreting the individual's maladjustment to environment."

"An internist is a physician who, through skill in these arts and sciences, is able to understand the individual and correct the maladjustment."

18 "A physician skilled in diagnostic methods possessing a comprehensive understanding of the etiology and pathology of disease with a knowledge of psychology, pharmacology, immunology and pathologic physiology adequate to enable him to prescribe a rational therapy."

19 "My inference is that the internist must base his practice on a primary, intimate knowledge of pathology on an accurate and extensive schooling in all the methods of diagnosis, and must be schooled in all therapeutic measurements except those involving surgery."

20 "In the clinics of Germany and Austria the professor of medicine is distinguished from surgery, has headed the department known as 'Innere Medizin'. The patients of

cupying his wards were believed to have 'innere krankheiten' or internal diseases requiring study of the function or disability of internal organs. I believe the term 'innere medicin' originated in the application of more refined methods of diagnosis before treatment, that is, the methods were carried further than was possible in other departments of the hospital or by the so called general practitioner of medicine who covered the whole ground, more or less, outside the hospital. The term internal medicine was the translation of Innere Medizin and as such has come into general use by physicians. It is interesting to note that the Standard Dictionary does not define internal medicine or internist. Dorland's American Medical Dictionary defines an internist as 'a physician who treats diseases of the internal organs' and internal medicine as 'that department which deals with diseases that cannot be treated surgically, medicine is distinguished from surgery.'

"An internist, as I understand the term, is one who has had special training in the refinements of diagnosis, and whose judgment has been founded upon extended experience in general diseases not requiring surgical relief. I acknowledge that the term internal medicine is difficult to define in a few words, and that it is confusing to many Americans. The field which it covers is fairly well understood on the European Continent. I have no suggestions as to better terminology."

GROUP V

In this last group a new factor appears. This is the conception of the internist as an integrator of all the facts discovered or discoverable in an individual case not only by himself but also with the help of any specialists, and the director of their proper application. This is the concept that is put to practical application in the proper working of group medicine.

21 "I share with you the difficulty of finding a satisfactory definition of an internist. Of course the term was introduced to distinguish the worker in internal medicine from the worker in so called external medicine. The former included the study of the body outside of the provinces that belonged to surgery whereas the latter included surgery, the surgical specialties and perhaps dermatology.

"Of course such a definition no longer holds, for the internist has adopted the biologic view of the organism that views each patient as a phenotype or realized person that has resulted from a succession of interactions of environment with the genotype. As a result of this biologic conception of the organism as a whole the internist has become the leader in general diagnostic studies in which complete analyses of all the systems of the body are made, after which the findings are synthesized into a multidimensional diagnosis with the various indications for therapy that this study reveals. As a rule internists, therefore, make much more careful and much more comprehensive studies of the organism and all its parts than do surgeons or the surgical specialists or even the so called specialists in medical domains (gastroenterology, cardiology, neurology, etc.).

"It seems to me that this recent development is a movement in the right direction and that probably all patients should be subjected to a general diagnostic study, for often the domain in which their complaints seem to be centered is not the domain in which the main trouble exists. There is no reason, of course, why a surgeon or a specialist should not conduct such general diagnostic surveys and make such multidimensional diagnoses if his training and experience fit him for it, but on the whole internists are, as a rule, better fitted by training and experience for the conduct of such general diagnostic surveys with subsequent integration."

22 "There should not be any such thing as 'internal medicine' because I think it is impossible to define. However, after laboriously considering the matter, I submit the following, unsatisfactory as it is:

"Internal medicine—the branch of medical practice characterized in distinctive measure by its aim at definitive treatment, advised by the practitioner in this branch, to be carried out by himself or others more specially expert, according to the patient's need, as re

vered by a relatively comprehensive diagnosis made by the practitioner in this branch, as a result of his correlation of his investigations, supplemented by those of any specially qualified consultants who may have been selected by him."

23 "An internist is a physician who limits his work to diagnosis and consultation. Filling as he does, a place which overlaps every other department of medicine, he correlates the work of other men and can sit in judgment on all procedures which are not technically surgical. He is essentially a helper and can treat on request."

"When he treats cases which come to him of their own volition he is merely a practitioner of medicine, with such deletions of patients as he individually debars."

24 "Concerning the definition of internal medicine and of an internist, I would say that you have asked a good one. I would find it difficult or impossible to express it in a few words. An internist in my mind is a physician who concerns himself with the diagnosis, prognosis, and treatment of all adults no matter what the complaint. I make that distinction in order to permit a subdivision of pediatrics. The treatment of some of these conditions that he will diagnose will necessarily be delegated to other specially trained physicians. Having found out that the patient has a brain tumor, he is naturally sent to a competent neurologic surgeon. Having diagnosed the abdominal tumor as due to pregnancy that patient goes to an obstetrician. Having found that the itch that troubles the patient is due to pityriasis rosea, he probably will be sent to a dermatologist. But if it is due to Hodgkin's disease he will probably look after it himself with the aid of the roentgenologist, etc. He may therefore treat a remaining group of patients that he feels competent to care for and even here he may again delegate part of them to special internists because of particular qualifications that they may have."

From a review of the preceding expressions of opinion on the significance of the terms Internist and Internal Medicine the difficulties of concise definition become obvious. Indeed, we are inclined to agree with those correspondents who feel that it is impossible to define internal medicine because it blends in to so many of the specialties.

After reviewing the above definitions contributed by leading thinkers throughout the country one would have considerable temerity in proposing a substitute definition. Internal medicine is in the last analysis a misnomer and therefore any attempt to define it must be to some extent patch work. The field of internal medicine does not deal exclusively with diseases of the internal organs although to be sure this may be said to be its major interest. Furthermore, other specialties such as urology, gynecology, proctology, roentgenology also deal with diseases of the internal organs.

There can be no specific definitive limitation with regard to the other specialties and it is readily understandable how different definers will disagree as to what of the medical specialties may be encroached upon. One will include neurology as a subdivision of internal medicine and another will exclude it. The limitations of internal medicine vary in the different diseases and even with individual cases. Iritis when due to local infection should properly be treated by the ophthalmologist but when it is syphilitic in origin its treatment falls within the domain of internal medicine. Peptic ulcer and cholangitis are not necessarily always surgical. One case will be treated medically, another surgically, even by the same physician. The internist may refer one case of dermatitis to a dermatologist while he may prefer to treat his own case of arsenical dermatitis and his own allergic dermatitis. The treatment of diabetes in children does not differ from that of adults.

It becomes apparent therefore that internal medicine cannot be defined or described precisely in terms of the extent and limitations of its domain.

By tacit consent it would appear that the term internist implies an individual with an unusually broad perspective, certainly broader than that of the more highly specialized practitioners, and possibly a keener insight into the problems of the patient, considered in terms of his entire economy as a unit, and its relationship to his environment. The internist views the patient as a whole and modifies his treatment for a specific ailment in accordance with his understanding of his patient as an economic entity.

As a consequence the terms integrator, correlator, advisor, consultant and director may properly be applied to some of the functions of the internist. On him falls the burden for deciding for or against therapy in other special fields such as surgery, the decision being reached by virtue of intimate knowledge of all the factors which might have a possible bearing on the results of such treatment. Among such factors are the presence of cardiovascular or renal pathology, tuberculosis, diabetes, mental instability and indeed, not infrequently the patient's domestic problems and even his financial status.

In the field of diagnosis the internist must be a man of wide vision and must keep himself as free as possible from pet theories and hobbies. In the field of therapy he must retain for himself considerable latitude as to what he shall elect to treat himself. A patient with arthritis is referred to an internist for study and whatever treatment may be necessary. During the course of search for focal infection a crowned tooth is observed, infected tonsils are recognized and pus is obtained from the prostate by massage. In this one case, the internist at once proves himself inconsistent. He sends his patient to the dental radiologist for x-ray of the tooth and to an exodontist for tooth extraction. He refers him to an otolaryngologist for tonsillectomy. By the rules of the game he should refer him once more to a urologist for prostatic massage and bladder irrigation. But he considers himself entirely competent to perform this therapeutic procedure and proceeds to do so. If the patient be a woman with an endocervicitis, he may send her to a gynecologist, or, if proficient, he may himself cauterize the cervix thus clearing up this focus of infection. Provided he is competent in the fields of therapy which he may undertake, the internist reserves for himself considerable latitude.

It is true that internal medicine overlaps nearly all of the specialties. This is an argument in favor of saying that internal medicine is not in itself a specialty. It is equally true that many of the specialties overlap, not only internal medicine, but likewise other specialties. The diseases are numerous in which a patient will wander from one specialist to another, finally finding the one who can give most relief. And with the same disease it is not always the same type of specialist who accomplishes best results in every case. Syphilis is treated by the dermatologist and by the internist. The toxemias of pregnancy are treated by the obstetrician and by the internist. Arthritis comes within the domain both of internal medicine and of orthopedics. Malignancy is treated by the surgeon. It is also treated by the

roentgenologist The same is true of fibroid tumors Examples might be multiplied

One very competent pediatricist always insisted that he was not a specialist but was a general practitioner among children

The anteroom of Sir Jonathan Hutchinson, one of the leading surgeons of his time, was filled at all times not only with surgical cases but with skin conditions, individuals with cardiovascular diseases, and in short the entire gamut of the medical diseases Sir Jonathan, once questioned humorously as to his definition of surgery, with a twinkle in his eye slapped his hand upon the table saying, "Surgery is anything that comes to Sir Jonathan Hutchinson" Possibly the average internist feels much the same way about internal medicine It has been said that an internist is just a glorified general practitioner Here is food for thought

The young physicians recently graduated from our leading medical schools, after completing their terms of internship, unless they have undertaken some one of the other specialties, designate themselves as internists Indeed, the ideal to be aimed at in medical education is to give each student such thorough undergraduate and postgraduate instruction that every practitioner will be in essence an internist This brings us back to the concept expressed in several of our contributions, that the good term physician is more appropriate and more descriptive than the term internist

Internist and Internal Medicine are misnomers Are there no better, more appropriate designations which may be used?

Physician has been suggested There is another term which has gained great popularity with the laity, but which is not in such good grace with the medical profession This is the term Diagnostician In its more common implication, it is a designation not to be desired The average layman thinks of a diagnostician as an individual who can give you a name for your malady, ticketing you, labeling you but who is little interested in therapy This lay understanding of the term has come about as a result the use of the so called diagnostician as a consultant After the consultation the attending physician rather than the consultant proceeds with treatment

Even within the profession this understanding of the term has been rather widely accepted Dorland's Dictionary which derives the word diagnosis from two Greek words meaning "to know apart," defines diagnosis as the art of distinguishing one disease from another In this sense diagnosis would be but the classification of diseases

But if we will go back to the original Galenic interpretation of the term we will find that it assumes decidedly deeper significance True, *dia* does mean "apart" but it has other translations It may be translated as "through" or "throughout" and indeed is so translated in connection with other words in Dorland's Dictionary In this sense diagnosis would mean "*to know through or throughout or thoroughly, to understand thoroughly*" When Galen said that it took him long to diagnose the pulse, that it does not merely rise and fall but expands and contracts he meant that it took him a long time to thoroughly understand the pulse

In this deeper sense Diagnosis and Diagnostician should find better favor. A diagnostician would be one with a thorough understanding of the patient's malady. This understanding would apply not only to *classification* but also to *treatment*. One cannot thoroughly understand a malady unless at the same time one knows the best remedies to apply and how to apply them.

The writer is not suggesting that the term Diagnostician be substituted for that of Internist, although aside from its recognized shortcomings it appears to be a more appropriate term.

But his understanding of the term diagnosis impels him to venture yet another definition of an internist. The following definition is not submitted as a composite of the twenty odd definitions recorded above nor as a substitute but merely as yet one more concept which it is hoped may stir up productive discussion on the subject. No claim for entire originality can be made since the same idea is expressed in several of the definitions which have been contributed above.

The points submitted in favor of this definition are (1) that it avoids some of the apparently weak points in certain of the definitions, such as relationship to internal organs, and the attempt to define the limitations of internal medicine in terms of the specialties, (2) that it includes the concept of an internist as being first of all a physician, (3) and the idea of correlation, and (4) that it is relatively brief and concise. True, it deals in generalities, but by now we must agree that the word is only susceptible to definition in general terms.

25 "An internist is a physician who through adequate training and experience has reached that stage in the art and science of medical practice, at which he possesses as thorough an understanding as possible of the nature of the maladies from which his patients are suffering, and is competent to prescribe appropriate treatment, or to advise the proper form of additional study or treatment, to be administered by another practitioner in some specialized field of medicine."

—W T V

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News and Notes

We read in *Science* that Dr John A Kolmer, professor of pathology and bacteriology in the graduate school of medicine of the University of Pennsylvania, was recently awarded the Mendel medal by Villa Nova College for his work in immunology. This is the first award of the medal, which was established to commemorate the work of Gregor Mendel.

Dr Benjamin S Kline of Cleveland will officially represent the American Society of Clinical Pathologists at the dedication of the new building of the Institute of Pathology of the Western Reserve University of Cleveland, Ohio.

The Committee appointed to look after local arrangements for the 1930 Convention of the American Society of Clinical Pathologists in Detroit, Michigan, are as follows: Dr Frank W Hartmann, Chairman, Dr Arthur L Amolsch, Dr O A Brines, Dr H E Cope, Dr O M Gruzhit, Dr Walter E King, Dr Clarence I Owen, Dr R G Owen and Dr C M Stafford, all of Detroit.

Dr D Schnaylor Pulford, Woodland Clinic, Woodland, California, has been appointed by our President, Dr J H Black, to serve on the Program Committee in the place of Dr Wm G Exton, Newark, N J.

A Columnist's Conception of a Technician

The morning mail brings a letter from a gentleman whose stationery proclaims him as a "Microscopist, Technologist, and Scientist." He asks for assistance in hunting a job. And him with a microscope!

The Office of the Society is in receipt of numerous invitations from cities throughout the country who are anxious to have our Convention in their midst. From one of these a

letter is addressed to the American Society of CRIMINAL Pathologists Comment We wonder whether this is an attribute of the profession or refers to the practice of forensic medicine

Letter From S C Dyke, DM, Wolverhampton & Staffordshire Hospital, Secretary of British Pathologists' Association

Wolverhampton, 2/9/29

Dear Dr Corper

Thank you for your letter of August 8th I take it as a great honor both to myself and to the British Pathologists' Association that your Society has seen fit to elect me a Corresponding Member Will you be so good as to convey to your Society the thanks of my Association?

I will see that copies of all transactions of my Association are forwarded to you and that you are kept posted as to any change in the secretaryship

I shall take the first opportunity of bringing your letter before the general body of my Association

I am

Yours very sincerely,

(Signed) S C DYKE

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No 2

CLINICAL AND EXPERIMENTAL

STUDIES ON AN ORGANISM ISOLATED FROM MALIGNANT TUMORS*

BY E W STEARN PH D P L STURDIVANT MD AND A D STEARN, PH D,
PASADENA CALIF

CULTURES of the organism here described have been obtained from

1 Filtrates from Berkefeld filter Grade w of (a) ground fresh tumor tissue and glands, (b) cultured ground tumor tissue, (c) old broth cultures showing the presence of gonidia and small bacilli and cocci, and (d) old agar cultures suspended in salt solution

2 Minced fresh tumor tissue cultured generally for one week in the case of adenotumors, but occasionally for as long as three weeks in the case of scirrhus tumors

3 Bloody peritoneal fluid from a patient with rhabdomyosarcoma, as well as from tissue from the same patient¹

4 Tumor mass from a rat (No 29) which had received five injections of the minute coccobacillus cultured from a human source during a period of five months

Medium for initial growth was a semisolid variety containing 500 gm beef heart, 5 gm NaCl, 15 gm peptone, 1 gm $KHPO_4$, 5 gm agar and 2 eggs per liter of water, and a liquid variety containing in place of the agar mentioned above, 1 gm gelatin

A more or less definite procedure was followed in the examination of tissues. Sections were aseptically removed and minced with sterile scissors and then planted to plates and deep tubes containing the medium. Other sections were ground with sand, emulsified and then filtered through a Berkefeld filter Grade w. If, after a week's incubation the tumor showed profuse growth, it was removed from the plate, ground with sterile sand, filtered and the filtrate planted. This procedure was to eliminate as readily as possible other

From the Laboratory of the Pasadena Hospital Pasadena Calif
Received for publication April 6 1929

organisms The plates containing the pieces of tissue and the filtrates were examined daily for evidence of the presence of the organism In most cases there was no observable change during the first forty-eight to seventy-two hours, and often the scirrhous type of carcinoma gave little evidence of the presence of an organism until the third week of incubation In all cases control plates and tubes were used and only media which had been incubated for from three to seven days were employed

All the tumors used in this study which have undergone comparable treatment are classified as to source in Table I

TABLE I
TOTAL NUMBER OF 33 TISSUES EXAMINED DISTRIBUTED AS FOLLOWS

MALIGNANT		PAPILLO	NONMALIGNANT
Adeno	7		
Scirrhous	10		
Medullary	5		
Basal cell carcinoma of skin	1		
Rhabdomyoma	1		
Carcinoma secondary to adenofibroma	1		
Totals	25	3	5

Of the 25 malignant tumors listed two, both medullary, were stored for an extended period in glycerin in the ice box to determine whether tissues so stored were favorably placed to preserve the organism during storage In neither instance was the organism isolated after storage There are, therefore, twenty-three malignant tissues which have been subjected to comparable treatment From these the microorganism was successfully isolated in every case except two, that is, it was obtained from over 91 per cent of the fresh tissues studied

Among the adeno type tissues there was one from the retroperitoneal gland, one from the ileum and cecum, one from the stomach and four from the breast The scirrhous and medullary types all came from the breast The nonmalignant tumors included 2 adenofibromas (breast), 1 colloid goiter, 1 uterine fibroid, 1 cyst adenoma (breast)

After the organism has accustomed itself to a saprophytic environment it grows readily at temperatures varying from 25 to 40° C, though now and then a strain shows a distinct preference for a high or a low temperature It gradually develops from a faint glistening growth to a deep sulphur yellow viscous growth, the appearance depending entirely on the conditions of development

Upon obtaining luxuriant growth after repeated subculturing, Orskov's single cell method of isolation was employed to insure single cell cultures On a soft, semisolid medium the organism develops as a viscous, slightly spreading growth When the medium is dry and solid, or when culturing takes place on potato plants, the growth is no longer viscous but shrivelled and flaky, and can readily be completely removed from the surface

Upon isolation from single cell cultures the life activities of the organism were studied Its most peculiar characteristic is its marked variation in

form under changed conditions. Several methods were used to determine the true character of its ontogenic cycle. One was to inoculate hanging drops, incubate them and then, after varying lengths of time stain the growth either by Nalanslu's intravital staining method or in the usual fixed smear. Another was the one of rapid transfer at intervals to various media, with microscopic examination at the times of transfer. In the ascitic fluid medium or in the beef heart liquid medium the organism assumes when grown under anaerobic conditions, the form of a minute coccobacillus (Fig 1)

Figs 1 to 6 show the dominant forms. The study of the life cycles of the strains was in no case attempted until single cell cultures obtained by the Ortolan technique had been secured.

The minute coccobacillus will when transplanted to a soft beef infusion agar, develop into a pointed rod (Fig. 2). If transplanted to broth this pointed



Fig. 1 —Coccobacilli from anaerobic culture

rod assumes the form of a greatly enlarged curved and sometimes hooked, bacillus (Fig 3). This curved rod continues to enlarge becomes beaded in appearance (whether stained or unstained) loses its motility and forms buds and branches (Fig 4). After an incubation of a week or occasionally longer, the branching forms disappear and instead we find the vacuolated nonstaining remains of these forms granules minute cocci, bacilli and greatly enlarged ovoid bodies. Such a culture when passed (through Grade w filter) gives a clear transparent filtrate which when transplanted to beef infusion media will yield a pure culture of a motile coccus (Fig 6). This motile coccus will retain its form at times for weeks but if transplanted to a dextrose medium, in which it will form acid the various rod forms develop within forty eight hours.

The organism will grow indefinitely either as a minute coccobacillus, a rod, or a coccus provided the substrates are not radically changed. It seems

that a strict adherence to a standardized environment will bring about a general character

It has apparently great powers of resistance to an adverse environment after it has become acclimated to cultural conditions. It survives storage for nine months on agar at refrigerator temperature. Due to its ability to

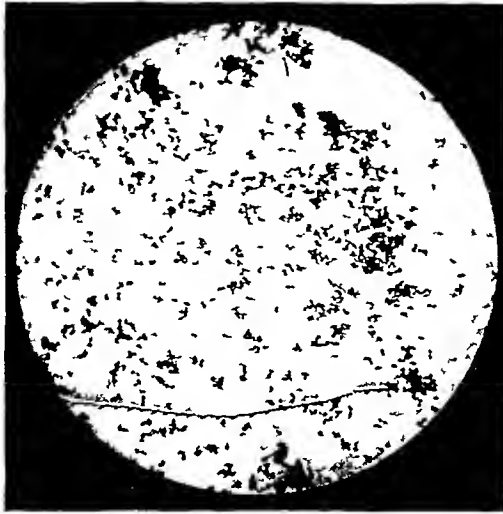


Fig 2—Pointed rod from solid medium

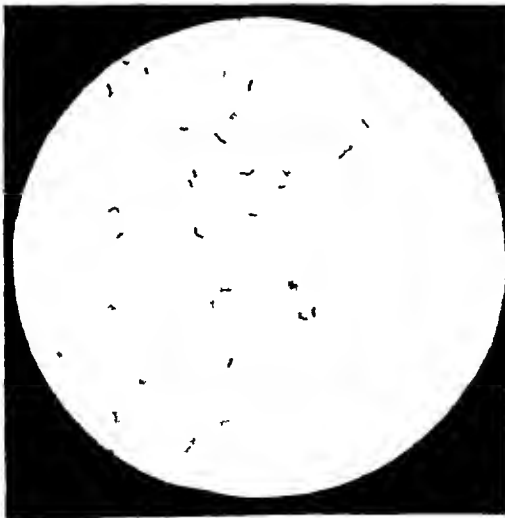


Fig 3—Curved rod from forty-eight hour lactose broth culture

form spores as well as gonidia it is remarkably well adapted to prolonged cultivation without the necessity of daily transplanting

Its general characteristics may be summed up as follows

- 1 It is actively motile at most stages of its development
- 2 The rod form develops spores which are found to resist a temperature of 70°C for ten minutes

3 The minute coccobacilli are gram negative though all smears show a few gram positive individuals

The rods and cocci are strongly gram positive

4 In certain stages of its development it is filterable, passing through a Berkefeld filter Grade w



Fig 4—Branching forms (one week old bouillon cultures)



Fig 5—Large oval bodies globular (possibly spore sacs) and small coccobacilli

The biologic behavior of the organism can be tabulated as follows

1 It ferments lactose, dextrose, sucrose, maltose and fructose, forming acid but no gas It should be noted that the ability to form acid from sugars is greatly reduced by prolonged artificial culturing

2 It reduces nitrates to nitrites

3 Its color on agar, gelatin and potato slants varies from a white to a sulphur yellow

4 It lacks the ability to hydrolyze starch

5 Only a slight amount of acid is formed in litmus milk even after prolonged incubation

6 Only a small amount of indol is formed from trypticized bouillon

7 It produces hydrogen sulphide

8 It is a facultative aerobe

9 It grows best in a medium whose reaction is adjusted to near neutrality

10 Slow liquefaction of gelatin is observable after ten days at room temperature, though gelatin liquefaction is at all times negligible

A unique and interesting property of this organism is its ability to produce crystals probably consisting mostly of magnesium ammonium phosphate,

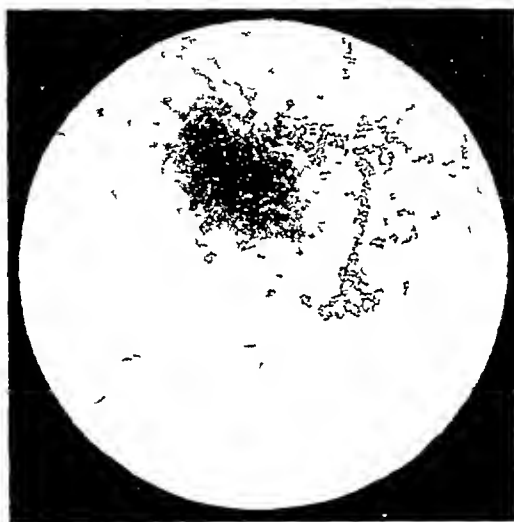


Fig 6—Cocci from starch plate

when the culture has been freshly isolated and is grown on beef heart media. Only one organism is listed by Bergey which has this ability to a sufficient degree to be so noted. The crystals when first formed by fresh cultures are overlaid by a yellow layer of the chromogenic organism, giving the appearance of sulphur crystals. The yellow layer could be easily washed off leaving white crystals. It has been found by Seudder³ and by us that many organisms, especially putrefying ones, can be made to produce such crystals in properly chosen media adjusted to proper reaction. Uninoculated media does not yield them.

A microanalysis yielded the following results

	PER CENT FOUND	THEORETICAL PER CENT FOR $MgNH_4PO_4 \cdot 6H_2O$
$Mg_3P_2O_8$	45.7	45.37
N	5.69	5.71
PO_4	37.9	38.73
Mg	9.85	9.91

In spite of this analytical data and the crystallographic identification of Seudder who concludes that "the significance of magnesium ammonium phosphate crystal production does not appear of importance at the present time," the authors have kept their minds open to other possibilities for the following reasons

1 A single preliminary experiment indicated some slight physiologic activity on the part of some constituent of the crystal which was similar to tyramine

2 The purified i.e., washed crystal when ignited, gave too much carbonization to be easily dismissed as being due to organic impurity for the medium, and too much to account for the rather good analytical checks. Furthermore, although little is known about such compounds there are many possible ones very similar chemically to magnesium ammonium phosphate but in which the ammonium is replaced by a ptomaine or substituted ammonia from the putrefaction of amino acids

3 During the formation of the crystals by the freshly cultured organism the odor of amines was sufficiently strong in the laboratory to cause general remark

It is therefore thought subject to further findings, that these crystals may be partly ordinary magnesium ammonium phosphate and partly some similar compound containing in place of the ammonia some ptomaine or some mixture of ptomaines since it has been found that many bacteria are not as efficient as most fungi in utilizing amines

Perhaps the chief interest in such an organism from a practical standpoint arises from a consideration of the immunologic tests with sera from patients suffering from carcinoma and their possible diagnostic value. So far only precipitin and agglutination tests have been studied

Blood from patients suffering from carcinoma was tested for the presence of precipitins. Normal human blood was used for control. The first tests were made with Berkefeld filtrates of three to six weeks old bouillon cultures. These filtrates gave results which were indicative of some specificity. It was found however that when this bouillon filtrate was treated with alcohol a precipitate formed. Therefore this precipitate was dried and dissolved in water and used in place of the original broth filtrate. The usual procedure for bacterial filtrates was employed. Citrated blood serum gave the most consistent results

Strong positive precipitin reactions were obtained with the sera from cancer patients, while those from normal persons remained negative. Blood from a person suffering from a multiple uterine fibroid gave a positive precipitin test

Though the opportunity for adequate appraisal of the value of these findings for diagnostic purposes has not been offered the results thus far warrant a thorough testing of such a precipitin reaction with some hundreds of sera

The data on the agglutination reactions are not extensive enough to warrant any generalizations. Normal human serum was used as control for all

strains at the time of each series study, and the tests were always repeated the following day. Likewise checks were run using *Bacillus coli* and *Staphylococcus albus*. It has never been found that a serum immunized against this organism possesses agglutinating ability for either *B. coli* or *staphylococcus*. Microscopic tests were used to supplement the macroscopic ones.

Sera from patients suffering from carcinoma possess comparatively high agglutinating titer for certain strains of the organism, though the same serum might possess no power of agglutination for other strains. For example the serum from a patient with epithelioma agglutinated readily in high dilution strains from adeno and sarcomatous tumors but did not agglutinate the strains from medullary tumors. Further work will tell whether there is a specificity between the strain and antibody of various tumor types, or whether the difference in agglutinability is related to the morphologic form of the organism.

Of the supposedly normal human sera used two gave slight agglutination reactions with one or two strains. One such was that of a patient suffering from double pyelitis, but this serum showed no reaction with either of two strains of *B. coli*. The other was from an old man whose history did not give evidence of malignancy.

On the whole the results indicate some difference in the agglutinability of the strains from the various types of tumors. The minute coccobacillus is agglutinated at higher dilutions of the sera than are the larger forms. The serum from rats immunized against the rat strain will agglutinate the strains isolated from human tumors at a comparatively high titer.

The problem of the pathogenicity of the organism remains open since the only animals so far used for experimentation were rabbits, guinea pigs, and rats.

The experimental results along this line may be briefly summarized as follows:

- 1 There was no noticeable effect on a rabbit after repeated injections of the minute coccobacillus into the blood stream.

- 2 Twelve guinea pigs were inoculated subcutaneously in the region of breast tissue. Autopsy (after death from overdose of ether) revealed either no evidence of the injections or only enlarged suprarenal glands. One guinea pig which had received injections under a ligature in the breast tissue died of bronchial pneumonia. Autopsy revealed enlarged inflamed lymph glands and a small tumor-like area in the breast which, upon histologic examination, showed early carcinomatous changes.

- 3 Sixty white rats were inoculated with the minute coccobacillus usually at intervals of three to four days for periods covering six to nine months. This comprised the maximum treatment, variation arising whenever the condition of the animal called for it. Injections into the groin or subcutaneously under the mammary tissue resulted in indurated areas which would usually disappear after several weeks. Rats which died or were killed by the anesthetic were autopsied. These revealed minute nodules along the

line of puncture which were shown histologically to be composed of inflammatory tissue

To the above summary we wish to append three particular cases of especial interest

1 The peritoneal fluid from a child suffering from rhabdomyosarcoma which at first showed the presence of various cocci but later on incubation, showed the rod form, was injected into the peritoneum of a rat. Nine injections were given over a period of three and a half months. The animal appeared so ill that it was killed with ether. Autopsy revealed gelatinous tumor areas, not encapsulated which were invading the lung tissue. The lung tissue proper showed congestion and infiltration with polymorphonuclear cells. Upon histologic examination the tumor was classified as chondrosarcoma.

2 One rat was given 5 injections of the minute coccobacillus into the area near the mammary tissue over a period of six months. The animal died and autopsy revealed that the omentum was filled with tumor like masses and that a tumor like growth was attached to the liver. A mass of tumors was found between the two lobes of the lungs. Histologic findings showed that all the tumors were forms of growth resembling carcinoma. Sections showed the presence of numerous coccoid bodies both intra as well as extracellular, easily distinguishable from the nucleus. Bloets from the tumor were aseptically removed and cultured according to the methods used for the human specimens. The minute coccobacillus typical of the human strain so far as we are able to determine was isolated in pure culture.

3 This rat strain of the microorganism was injected intraperitoneally into 8 rats. Six rats all comparatively young showed no effect at the end of two months. Two older rats which had received 6 injections died of narcosis. Both were autopsied. One showed a markedly thickened omentum and chronic inflammatory tissue. The other showed that the stomach, omentum and part of the small bowel were matted together by dense adhesions. A dense mass in the liver, and areas resembling milium tubercles were found on the upper surface of the lobe of the liver. Histologic examination showed a large caseating mass surrounded by a zone of polymorphonuclear cells. In the caseated area there was a clump of large epithelial cells having no regular arrangement and surrounded by a zone of polymorphonuclear cells and cellular debris. The bowel wall was markedly thickened and inflamed. Nothing definite as to the origin of the cells could be determined.

It may be of interest to state that Dr James Young of Edinburgh has very recently been kind enough to examine a set of slides submitted by us showing the various forms of the organism. In a private communication he has stated that he has "no doubt that you are dealing with the same organism as I have described," the reports of which he has published in a series of articles.⁴

A preliminary report on this organism was published by us and a re-

port of the methods used in an attempt at its classification has appeared in the *Journal of Bacteriology* *

The authors wish to acknowledge indebtedness to the Laboratory of Preventive Medicine, University of Missouri, and to its director, Dr M P Ryvenel

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THE EFFECT OF COLLOIDAL AND CRYSTALLOIDAL METALLIC COMPOUNDS IN NUTRITIONAL ANEMIA OF THE RAT*

BY ALFRED GOERNER, M.D., BROOKLYN N. Y.

IN THE course of experiments on the effect of various metals in conjunction with iron on the increase of hemoglobin in anemic animals we had occasion to try small quantities of manganese salts and found that these had a noticeable action in the production of hemoglobin, in fact, they were second only to copper in this respect. Zinc and aluminum salts were also tried, but there was no appreciable difference between the hemoglobin producing power of these salts when added to iron and that of iron salts alone. Colloidal solutions of some of these metals were also employed.

EXPERIMENTAL

To produce anemia in suitable animals white rats were put on a diet of powdered milk and distilled water. Animals weighing from 50 to 60 gm were chosen. This is a modification of Waddell and Steenbock's method.¹ As an example of the effect of this regime on young rats, Table I shows the hemoglobin as determined with a Newcomer hemoglobinometer in a series

TABLE I
DIET: POWDERED MILK AND DISTILLED WATER

TIME IN WEEKS	AVERAGE Hg GM PER 100 C.C.	PER CENT OF ORIGINAL Hg	NUMBER OF ANIMALS
0	14.76	100	24
5	7.34	50	10
7	5.57	38	8
11	3.83	26	3

This agrees well with Waddell and Steenbock's results (loc. cit.).

After the animals had been rendered anemic they were given the solutions of metallic compounds in addition to the powdered milk and distilled water. Controls were kept on the original diet producing the anemia.

TABLE II
EFFECT OF IRON AND MANGANESE SALTS ON ANEMIA

WEEKS	GM Hg PER 100 C.C.	PER CENT OF ORIGINAL Hg	NO. OF RATS
0	6.72	47	6
2	11.65	81	6
4	15.80	109	6
6	16.85	117	6

Table II shows the effect of traces of manganese in combination with iron on the hemoglobin of anemic animals in a series in which the two metals were

* From the Department of Biological Chemistry, Long Island College Hospital, Brooklyn, N. Y.

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given as solutions of crystalline salts (copper free), and in quantities of 0.5 mg of each metal per day. This was added to the ordinary diet of powdered milk and distilled water. Controls receiving no additions of these metals became more anemic and died. Another series of controls receiving only an addition of iron salts showed that this was capable of preventing a further loss of hemoglobin while on the anemia-producing diet but was not effective in increasing the hemoglobin.

The addition of these metals increased the hemoglobin far beyond the normal average, as could be seen by the increased color of the eyes and skin. In the above series iron and ammonium sulphate and manganese sulphate were used, but other salts had the same effect.

When zinc salts were added to iron salts, there was no such result, the combination being no better than iron salts alone. The same can be said of aluminum salts. Copper salts were better than manganese, in that smaller quantities were capable of producing the effect.

However, when colloidal solutions of iron and manganese were used as additions to the "anemia diet," the animals showed not only an inability to increase the hemoglobin but this actually decreased with the same rapidity and fatal result that followed in the case of the untreated controls. It will be remembered that giving crystalline iron salts alone resulted in no further loss of hemoglobin nor increase of it, but rather in stabilizing the amount found when the animal was put on the amended diet. The number of animals

TABLE III
EFFECT OF COLLOIDAL IRON AND MANGANESE ON ANEMIA

WEEKS	GM Hg PER 100 C C	PER CENT OF ORIGINAL Hg	NO OF ANIMALS
0	7.08	47	8
2	5.24	35	8
4	3.12	21	4
6	2.81	12	2

surviving depended on the degree of anemia at the time that the iron salt was added. When, however, colloidal iron and manganese were added, the anemia progressed rapidly.

The series of animals shown in Table III received 0.2 mg of iron and 0.1 mg of manganese metal per day. The action of colloidal copper and iron in

TABLE IV
EFFECT OF COLLOIDAL COPPER AND IRON

WEEKS	GM Hg PER 100 C C	PER CENT OF ORIGINAL Hg	NO OF ANIMALS
0	5.46	36	6
1	4.97	33	6
2½	4.81	32	6
5	3.01	20	2

amounts which have hemoglobin-producing powers when given in the crystalline state is shown in Table IV.

To ascertain whether colloidal iron, copper, and manganese solutions had power to prevent anemia in rats when these were put on the "anemia diet,"

all three metals in the form of colloidal hydroxides were administered daily to rats weighing between 50 and 60 gm and having the normal amount of hemoglobin

TABLE V
EFFECT OF COLLOIDAL IRON, MANGANESE AND COPPER IN PREVENTING ANEMIA

WEEKS	GM Hg PER 100 CC	PER CENT OF ORIGINAL Hg	NO OF ANIMALS
0	11.04	100.0	11
2	12.01	85.6	11
4	9.23	65.7	10
6	7.24	51.5	4
9	3.10	22.1	2
CONTROLS			
0	14.15	100.0	6
2	12.07	85.3	6
4	9.66	68.2	6
6	7.20	50.9	3
9	3.01	21.2	1

The decrease of hemoglobin in both sets of animals is very similar

RESULTS

Crystalline salts of manganese as well as of copper are capable of increasing the hemoglobin of anemic animals when these salts are added to iron salts. Pure crystalline iron salts in solution have not the same pronounced effect.

Crystalline salts of zinc and aluminum when added to iron salts fail to show any marked hemoglobin increase.

Colloidal solutions of manganese and copper in the presence of colloidal solutions of iron have not this hemoglobin producing power when administered in quantities comparable to the crystalline salts, nor have the former the power of preventing the reduction of hemoglobin when the animals are placed on a diet capable of producing severe anemia.

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DESCRIPTION OF THE ORGANISM

The sputum of the patient was thick and yellowish gray in color. On microscopic examination (February 7, 1929) it was found to contain a large amount of pus and many bacteria but no tubercular organisms. On re-examination of the sputum one week later a large number of definite yeasts were observed. Three months later the sputum still contained many pus cells and bacteria. However, only after a short period of incubation did it give a definite yeast odor.

The feces showed occult blood, pus cells and many yeast cells. The urine was negative.

For the isolation of the *Monilia* washed sputum was planted on glucose agar and glucose broth. After a pure culture was obtained the organism was grown, for purposes of identification, in the following media: Maltose agar,

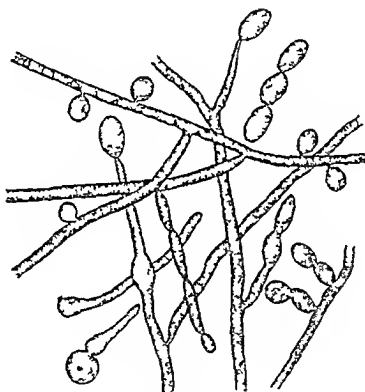


Fig. 1

litmus lactose agar, gelatin, glycerine agar, litmus milk, 1 per cent glucose, maltose, sucrose, lactose, levulose, salicin and mannitol broths.

Morphology.—At the end of ten days a large ovoid yeast cell was found in all broth cultures. The cultures contained definite nuclei and the cytoplasm was nonvacuolated. After seven days in all liquid media there were found filaments of from 6 to 15 cells in length with an abundance of spores. Fig. 1. On solid medium yeast forms persisted after ten days.

Reproduction.—Four methods of reproduction were observed. The simple yeast-like forms reproduced by budding and blastospores. Ascospores were not found. The filamentous forms produced arthrospores terminal and lateral conidia.

Staining Reactions.—Both yeast-like and filamentous forms stained readily with simple anilin stains and were gram positive. They were not acid fast.

Cultural Characteristics.—On all solid media the *Monilia* produced a thick, white, elevated creamy growth. Gelatin was not liquefied. It rendered milk

alkaline In one per cent glucose, maltose, levulose and sucrose bouillons abundant acid and gas were formed In glucose and maltose bouillon it grew with a distinct collar formation In no liquid medium was a pellicle seen, but after ten days the broth cleared with the formation of a heavy sediment

The cultural characteristic agrees with that of *Monilia metalondinensis* as described by Castellini⁴

ANIMAL EXPERIMENTATION

The pathogenicity of the strain was tested by inoculation into a 250 gm guinea pig It formed neither an abscess at the point of inoculation, nor was it pathogenic for the animal

PROGRESS OF THE PATIENT

The patient improved under symptomatic treatment After three weeks the cough was absent The chest was negative save for coarse râles on deep inspiration Six weeks later the patient complained of a slight cough, especially in the morning The skin was moist and blood pressure was 122/78 with a pulse of 90

Diagnosis—The condition was diagnosed as one of acute general respiratory infection of the influenzal nature However, the laboratory findings ultimately led to a diagnosis of a respiratory fungus infection

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THE EFFECT OF GUANIDINE COMPOUNDS ON UNANESTHETIZED DOGS

BY RALPH H MAJOR AND C J WEBER, KANSAS CITY, KANSAS

ALTHOUGH the majority of observers find that guanidine salts produce elevation in blood pressure, there are occasional accounts of negative results. Dominguez,¹ in a series of observations made upon nonanesthetized rabbits, has found that the blood pressure in these animals instead of being elevated, frequently falls. One of the interesting points which the work of Dominguez raises is whether unanesthetized animals behave differently from anesthetized animals.

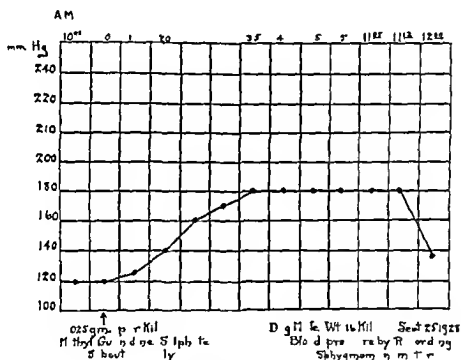


FIG. 1

We have frequently injected methylguanidine sulphate in anesthetized rabbits and produced marked elevations in blood pressure. We have not employed, however, doses as large as those employed by Dominguez since in our experience 0.1 gm per kilo of body weight in anesthetized rabbits is a toxic dose and usually produces a fall in blood pressure. In doses of 0.05 to 0.03 gm per kilo we have, however, very often seen striking rises. The rabbit is, however, not an animal very well adapted to blood pressure studies and shows some reactions unlike those encountered in other mammals. A striking instance of this is its behavior to histamine a substance that produces a marked fall in blood pressure in dogs, cats, and man, but causes a striking rise in blood pressure in rabbits. We have found in our experimental work

From the University of Kansas School of Medicine Kansas City Kansas
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on blood pressure in rabbits, that extremely variable results may be obtained in certain rabbits

To study the question whether anesthesia modifies the pressor effect of guanidine salts, we have carried out a series of observations on dogs, using the Tyco's recording sphygmomanometer for our estimations of blood pressure. In some animals, extremely good tracings may be obtained with this instrument, and we employed only those animals in which tracings of this kind were obtained. Experiments were carried out on 6 dogs and the results of two experiments shown in Figs 1 and 2. These charts have been compiled from data obtained by the use of the recording sphygmomanometer. While with this instrument it was not always possible to be sure whether our readings were correct within 5 or 10 mm, yet marked changes such as those following the injections, are easily noted.

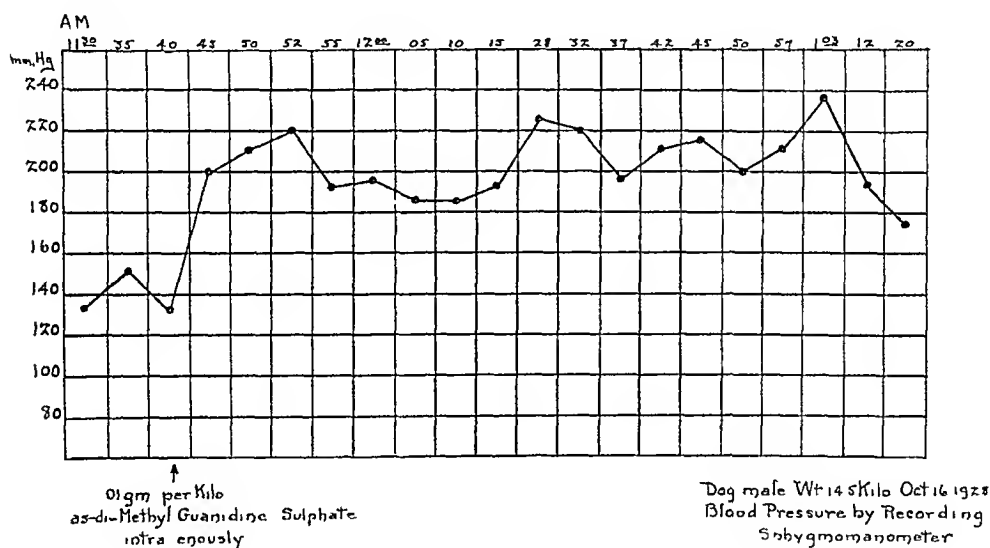


Fig. 2

We have found by this method that a dose as small as 0.025 gm per kilo of methylguanidine sulphate or 0.01 gm per kilo of as-dimethylguanidine sulphate produces very striking elevation in blood pressure. We have found that in the unanesthetized animals a dose of 0.1 gm per kilo (a dose which we have employed frequently on the anesthetized animals), often produces very marked symptoms of intoxication with rapid breathing and frothing at the mouth.

In the dog, we have not found that 'the circulatory effect of methylguanidine salts used is inconspicuous when compared with the picture of the intoxication,' as Dominguez noted in the rabbit, but on the contrary have found that the blood pressure can be raised very strikingly before any symptoms of intoxication appear. We have also in a few carefully controlled experiments noted the same thing in human beings in whom we have produced an elevation in blood pressure by gradually increasing doses, the dosage never being pushed to the point of producing unpleasant symptoms or intoxication."

One of the striking features of our experiments was the demonstration that the dose necessary to produce elevation in blood pressure was much smaller in the unanesthetized animal than in the dog under ether

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STUDIES IN THE PHARMACOLOGY OF LOCAL ANESTHETICS*

I TERMINAL INFILTRATION OF LOCAL ANESTHETICS BY THE DERMAL WHEEL METHOD ON GUINEA PIGS

BY C L ROSE, INDIANAPOLIS, IND

PITTENGER¹ has evaluated local anesthetic substances by subcutaneous injection on the backs of dogs Meeker,² repeating Pittenger's work on procaine and cocaine, but substituting intracutaneous infiltration for subcutaneous injection, points out that subcutaneous tissues are not sufficiently sensitive for this type of experiment Sollman³ and Meeker⁴ have reported on tests in which dermal wheals have been made by intracutaneous injection on man None of these procedures have lent themselves readily to routine, and it has been necessary to seek further for a more suitable laboratory method

Guinea pigs are found to be ideal for this kind of work They are easily handled, inexpensive, respond readily to weak stimuli, and with short rest periods between injections, may be used many times A method, which has become routine procedure in evaluating new local anesthetic compounds, has been developed, and a study of its technique is offered with the hope that it may be useful to others working in this field

Pigs weighing from 300 to 350 grams are the best for this test After the hair has been clipped from their backs, they are placed in small, open-topped wire baskets (4.5 by 7.75 by 4.5 in) Injections are made with a 1 cc glass tuberculin syringe fitted with a No. 24 gauge needle In making the injection the needle point must first be forced completely through the external layers of skin, and then turned outward and carried into the skin from the inner side far enough to prevent leaking This procedure may seem to be unnecessarily complicated, but due to the extreme toughness of the epidermis on the pig's back, this method has been found to be the most efficient for obtaining an *intracutaneous* injection

The margin of the dermal wheal thus formed is marked with ink The marked area is stimulated within one minute after injection, and once every minute thereafter until the local anesthetic has taken effect It is then stimulated at two minute intervals up to the time when the area becomes sensitive again This is the end-point of the experiment Stimulus is supplied by a Harvard inductorium connected to two dry-cell batteries in parallel, each with a capacity of 1.5 volts The secondary or movable coil is fixed in such relation to the core that the stimulus is just perceptible when the electrodes are applied to the flexor surface of the forearm of the operator This stimulus when applied to an unanesthetized area on the pig's back, is just sufficient to cause the animal to move

*From the Eli Lilly Research Laboratories Indianapolis Indiana
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The standard weight of the animals was fixed after an experiment, the results of which are shown in Table I

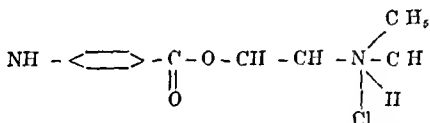
TABLE I
ONE PER CENT SOLUTION OF PROCAINE GIVEN IN DOSES OF 0.1 CC

PIG WEIGHT	PIG 1	PIG 2	PIG 3	AVERAGE
300 gms	22	25	23	24.0
400 "	24	18	23	21.6
500 "	16	21	15	17.1
300 "	22	26	24	24.0
400 "	23	21	21	21.6
500 "	16	17	19	17.3

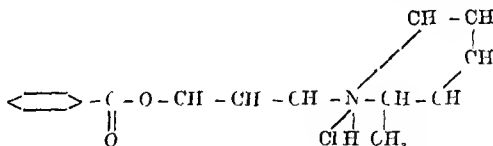
Duration expressed in minutes

The same lot (60474) of procaine in the same concentration and dose produced in dermal wheals on men an average duration of 24.5 minutes. This coincides with results shown in Table I for two groups of pigs each individual of which weighed 300 grams

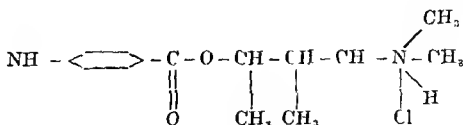
For purposes of comparison, three compounds, β diethyl amino ethyl para amino benzoate hydrochloride (procaine),



1-(2 methyl piperidino) propyl benzoate hydrochloride (our number 33) (4)



and p amino benzoyl dimethyl amino methyl butanol hydrochloride (tutocaine)



were injected in 0.1 cc doses of 1.1, 1.0 and 0.9 per cent solution with results as shown in Table II

From Table II, it can be seen that a difference in concentration of local anesthetic solution of less than 10 per cent may be detected by this method

The rabbit cornea method, described by Schmitz and Loevenhart⁶ and others,^{7,8,9} has become established as a standard for evaluating compounds, with local anesthetic properties when applied to mucous membranes. It is to be recognized that tests involving in the one case intracutaneous tissue, and in the other, mucous membrane may differ widely in results on the same

compound Because of its importance, the Schmitz and Loevenhart method is compared with the pig method, using tutocaine, a compound which produces local anesthesia by both infiltration and topical application

The pigs show greater regularity in response than rabbits when treated with this compound (tutocaine)

TABLE II

A COMPARISON OF THREE LOCAL ANESTHETIC COMPOUNDS, THREE PIGS USED WITH EACH DILUTION

COMPOUND	PER CENT SOLUTION	DURATION IN MINUTES
Procaine	0.9	20.0
	1.0	24.2
	1.1	27.6
No. 33	0.9	27.0
	1.0	32.0
	1.1	43.0
Tutocaine	0.9	15.0
	1.0	25.0
	1.1	35.0

TABLE III

A COMPARISON OF THE RABBIT CORNEA METHOD WITH THE GUINEA PIG METHOD, USING TWO GROUPS OF RABBITS AND PIGS. THREE ANIMALS CONSTITUTE A GROUP

PREPARATION	PER CENT SOLUTION	DURATION IN MINUTES	
		RABBIT CORNEA METHOD DOSE—0.25 c.c.	GUINEA PIG METHOD DOSE—0.1 c.c.
Tutocaine	0.9	26	16
	1.0	26	24
	1.1	38	36
Tutocaine	0.9	33	15
	1.0	35	25
	1.1	53	35

The differences in duration for the pigs are proportional to the differences in concentration of the local anesthetic. This is not true for the rabbits in the same range, showing them to be less sensitive than the pigs to changes in concentration. This must not be taken to mean that either of these methods may be used to the exclusion of the other. Many compounds are effective local anesthetics when introduced by the intraocular method, but have little or no effect on mucous membranes.

The animals should not be too closely confined or fastened when being used for testing. Such conditions lead to excitement which quickly results in fatigue. This was shown very clearly by testing a group of pigs early in the morning and then fastening them in an extended position to an animal board.

TABLE IV

A COMPARISON BETWEEN PIGS WITH AND WITHOUT INDUCED FATIGUE

PREPARATION	PER CENT SOLUTION AND TIME	DURATION
Tutocaine	1% solution before fatigue	25 minutes
	1% solution after fatigue	70 minutes
Tutocaine	1% solution in morning	28 minutes
	1% solution in afternoon	26 minutes

for two hours. At the end of this time the pigs were released and tested again with the same solution. A check experiment was carried out in which another group of pigs was tested in the morning and again late in the afternoon omitting the fatigue producing process. This eliminated the possibility of the time of day affecting the duration of anesthesia in a manner like that of fatigue. The results of these experiments are shown in Table IV.

SUMMARY

1 A description is given of a method for terminal infiltration of local anesthetic solutions by dermal wheals on guinea pigs.

2 The results obtained by this method are affected by the following factors:

- a Age and weight of pig
- b Amount of local anesthetic
- c Type of local anesthetic
- d Fatigue

3 The method gives results which correspond closely to those obtained by the dermal wheal method on man.

4 It is accurate to within less than 10 per cent difference in concentration of local anesthetic solution.

5 It is more sensitive than the rabbit's cornea method.

6 It is believed that the rabbit's cornea method and the guinea pig method together, give results which constitute a reliable index of the value of a local anesthetic.

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STUDIES IN THE ALIMENTARY TRACT OF MAN*

IV THE REFLEX EFFECT OF HEAT AND COLD UPON GASTRIC RESPONSES

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INTRODUCTION

IN OUR previous communications^{5, 9, 10} we have shown how training stabilizes the human stomach so that its response to a definite stimulus becomes constant in type. We have defined the conditions under which the experiment must be carried out and have made clear the success with which extraneous influences may be minimized.

As soon as we assured ourselves that it is actually possible to stabilize human gastric responses we carried out our plans for the investigation of the simplest gastric reaction pattern, namely, that of milk. We used milk rather than water because it is the most general mammalian food and could serve as a basis for the investigation alike of adult and young stomachs. For contrast with the milk response we determined upon buttermilk, thinking in terms of the buttermilk of our childhood from which butter had actually been churned. It was only in the course of our investigation that we discovered, to our chagrin, that buttermilk today is merely artificially made lactic milk. In consequence of this fact we have used that type known as "plain lactic" to form a vehicle contrasting with milk.

We were careful to keep the temperature of both milk and buttermilk at 70° F. This is approximately room temperature and is the temperature at which milk is generally palatable. Initial experiments showed us that a variation of five degrees or so above or below our standard temperature is of no practical significance.

We have also made it plain that along with the conditions of the experiment and the temperature of the vehicle, the amount of the meal must be standardized. We work with a 5-ounce meal which, no matter what the degree of stomach activity, is practically all passed through the pylorus in an hour. It consists of four ounces of vehicle by volume to which are added thirty-three grams of barium sulphate by weight. A larger meal is not called for because, although the reaction pattern induced by it is the same as that stimulated by the small meal, its time relations are altered.

Our observations have now been carried out upon two successive series of trained stomachs each of thirty-six individuals. On one of these series the investigation was repeated six months later. The constancy of result gives us confidence in the reality of the distinctions in reaction pattern.

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A milk meal produces a shadow area of smaller dimensions than that induced by buttermilk. The elongation and lateral distension of the shadow so well seen after buttermilk are much less evident after milk.

Peristaltic activity commences within two minutes after ingestion of a milk meal, but starts immediately after ingestion of a buttermilk meal. The peristaltic waves induced by milk are of low amplitude and indeed may be represented by a mere shimmer of the greater curvature. They progress at intervals of approximately twenty seconds whereas buttermilk waves which are far more massive and usually involve both curvatures, perhaps punching off the entire lumen of the pyloric canal or even of the distal pyloric vestibule, follow each other at intervals of approximately fifteen seconds.

The third distinction is in the character of passage. A milk shadow in the pylorus and duodenum resembles a puff of smoke whereas a buttermilk shadow is denser and larger. In spite of this distinction there is no difference in emptying time. It is unusual to see even an imperfect cap shadow at first after ingestion of a milk meal although after the lapse of an hour when the gastric contents are negligible and no passage can be observed, there may be a full cap. After buttermilk, on the contrary, a full cap rapidly develops and duodenal contractions are powerfully stimulated with a resultant mooniform shadow.

With the characteristic reaction pattern induced by milk clearly before us we have felt justified in carrying out further experiments to determine the effect of other agents upon gastric responses. In this paper we shall present the effects of heat and cold.

It is apparent that to keep the conditions of the experiment uniform we must use the reaction pattern of milk at 70° F. as our basis. Now if the milk be heated or cooled and definite changes in reaction pattern occur we are left in doubt as to whether these are due to a direct stimulation of gastric musculature or to a reflex stimulus. Hence we have established two sets of experiments. In the first the temperature of the milk was changed; in the second the milk temperature was kept constant and heat or cold applied to the abdominal wall.

This is not the first time that the influence of heat and cold upon gastric response has been investigated. In 1920 two German workers, Weitz and Sterkel,⁶ made fluoroscopic tracings of the stomachs of normal young adult men. The examinations were made at a room temperature of between 50° and 36° F. which the authors called cold and again at 68° F. or above which they defined as warm. The meal was a large one and was always at about 100° F. The results obtained were quite varied but there was a fairly constant difference in position of greater curvature. In the 'warm' stomach tracing the greater curvature lay at a lower level than in the 'cold' tracing.

In our experience a meal at such a temperature would counteract the slight effect of such differences in room temperature and might readily produce a nausea in the subject. The conditions of experiment were not carefully described and it is impossible to extract any definite gastric behavior pattern from the details given. We find ourselves unable to accept all the conclusions drawn from these observations but we would emphasize the significance of

two The first is the effect of cold in producing a stomach shadow which is narrowed and only slightly shortened The second is the inference that the effect of cold is reflex and not direct

In a much more recent paper Atkinson² has described the effect of heat and cold on gastric activity in dogs He applied hot and cold packs to the abdomen over periods of half to one hour Water at 140° F and at near 32° F was introduced through a fistula in the stomach The dogs were not anesthetized and erratic results were obtained The author believes that the importance in Man of the effect of swallowing hot and cold fluids has been exaggerated He concludes that external application of cold results in diminished gastric tone, while heat increases tone, but the swallowing of either hot or cold water reduces tone and diminishes peristalsis

We find that the introduction of water into the stomach produces a gastric behavior pattern different from that of milk and we do not believe that the results obtained with water can be interpreted as characteristic for milk or any other meal The observations which we are about to record lead us to believe that the results obtained by Atkinson after external application of heat or cold may be due in part to other unrecognized influences at work in the animals

It would be wearisome and wholly profitless to set forth further summaries of previous semi-clinical work done upon gastric motility, whether upon experimental animals or upon Man Those who desire to acquaint themselves with the historic aspect of this problem may consult the references in Atkinson's article above, or in the articles by M'Crea, M'Swney, Morison and Stopford³ or, much more profitably, the recent edition of Alvarez' book¹ Nevertheless they will not find in any of the previous semi-clinical work those carefully controlled conditions in which alone the gastric patterns can be analyzed with the confidence born of an ability to reproduce a reaction pattern at will by re-establishing the proper conditions In omitting reference to such work it must not be assumed that we either ignore it or are ignorant of it (see, for example⁴ *) So confused have statements become, whether relating to anatomy or physiology of the stomach, so bound are they by tradition, by inadequate observation or imperfect acquaintance with the problem that we prefer to make a plain record of our own observations untrammelled by those of others, but controlled step by step, with a completeness not previously attainable

CONDITIONS OF THE STUDY

The total number of trained stomachs for this study was thirty-one Two others which were used in the initial trial observations were omitted from the final series The observations made on the two however are quite in accordance with those on the thirty-one

After examination of the records and consideration of the conditions of experiment as indicated by the two trials, the following was the plan developed and rigidly carried out in the final study

The observations were made in October 1927 upon Sophomores, this grade of students and time of year being deliberately chosen from experience de-

talled in our former papers.^{1,2,4} Each experiment lasted two successive days and the students were grouped in fours. On the first day two students took a milk meal followed by a buttermilk meal an hour later; the other two students took a buttermilk meal first and a milk meal an hour afterwards. Thus the first day was spent making sure of the stabilized pattern of the gastric response. On the second day the students followed a routine dictated by a three folds carefully drawn up to ensure equal distribution of various possible combinations in nature and order of experiment. By this means we obtained the following series:

I	II	III	IV
1st cold	1st heat	1st cold	1st heat
1st cold	1st heat	1st cold	1st heat
V	VI	VII	VIII
1st cold	1st heat	1st cold	1st heat
1st heat	1st cold	1st heat	1st cold

For the hot drink a regular 5 ounce milk meal was heated to 110°F and for the cold drink a similar regular milk meal was cooled to 45°F . For the indirect application of heat and cold the student sat quietly for a while with a hot water bag or an ice pack wrapped in a towel and placed next the side of his anterior abdominal wall. As soon as he could bear the heat or the cold he stopped the appliance in place and wrapping his outer garments about him was free to move about the laboratory. We were particularly anxious that he should not be down for fear of introducing another factor into our observations. The after effect of a marked change of posture is considerable in some people, at least upon the intestines, and we have no doubt of observations upon possible effect on the stomach. The hot bag or cold pack continued in contact with the abdominal wall for forty five minutes and was removed immediately before the ingestion of a regular 5 ounce milk meal at 70°F .

THE INFLUENCE OF HEAT AND COLD UPON GASTRIC DIMENSIONS

Table I sets forth the results of our cinematographic study. Even the most painstaking analysis however given a very imperfect impression of the differences in gastric response as demonstrable by the fluoroscopic screen.

Average width as measured in the gastric tube at the base of the Mager's blase; average height is the projection distance measured vertically parallel with the vertebral column, from the summit of the Magerblase to the lowest point on the greater curvature in the pyloric vestibule. The area is estimated by the planimeter of which the record as we have previously shown² must be accepted with a possible error of 11 percent.

It is at once evident that the experimental changes induced in dimensions are more striking when heat or cold is applied to the abdominal wall than when the gastric mucosa is directly stimulated. Indeed there may reasonably be doubt whether any real distinction exists at all if the several figures are looked at separately. It is in their entirety that the results carry abiding force. Bearing in mind the very great individual variation in dimensions of the stomach even when uniformly of experimental conditions has been most excellently attained, it is remarkable that there should occur such obvious differences of the averages.

FACILITATION

We have previously demonstrated, in our description of experiments upon the reaction patterns of milk and buttermilk, that the characteristic features of gastric response are intensified if they be stimulated after the exhibition of a contrasting reaction pattern. Thus the distinction between the dimensions of gastric shadow induced by milk and buttermilk is greater if a series of students be used who have had meals respectively of buttermilk and milk an hour before. The latter part of Table I shows that the same facilitation of

TABLE I

1 INFLUENCE OF HEAT AND COLD ON GASTRIC DIMENSIONS				
	NO	AVERAGE WIDTH	AVERAGE HEIGHT	AVERAGE AREA
Regular Milk	31	50 mm	206 mm	11011 sq mm
Internal Heat	15	52	199	10088
External Heat	15	47	181	8944
Internal Cold	15	48	210	10538
External Cold	16	48	202	9898

2 INFLUENCE OF ORDER OF APPLICATION			HEAT AND COLD
			AVERAGE AREA
Regular milk meal	first		11372 sq mm
	second		10673
Internal heat	first		10476
	second		9449
External heat	first		9458
	second		8419
Internal cold	first		10311
	second		10764
External cold	first		9551
	second		10245

response can be elicited after heat and cold. It is, as a matter of fact, very important that our results should be subjected to this further analysis. One may object that the series are now very small, each consisting of but four students, and that the harmony of result is a mere coincidence. We do not expect to have the final word in this matter but are willing to have our findings reinvestigated by any worker who will give the same meticulous care which we have given to the experimental conditions. We feel sure that he will attain like results. (Since the above was written we have repeated the experiments on another series of similar number and have obtained identical results.)

In the scrutiny of these figures detailed in the latter part of Table I we should bear in mind the possibility of an error of 6 percent which we have established for all planimetric records of gastric shadow area. The possible error for first meal shadows approximates 750 sq mm and for second meals 650 sq mm. Now we find that in first meals the average for internal heat is 896 sq mm less than that for regular milk and for second meals the difference is 1224 sq mm. While then there may be reasonable doubt regarding the genuineness of the difference based on first meals there can be no doubt at all of

the difference shown by second meals. For external heat the reduction in average for first meals is 1914 sq mm and that for second meals 2264 sq mm. These differences are beyond dispute. The effect of heat is quite clear.

Now when we examine the result of cold we find a very different relationship. It is the first meals which show a difference, not the second meals. Subtracting the average areas of first meals from those of the corresponding standard milk meals at 70° F we obtained 1061 sq mm for internal cold and 1821 sq mm for external cold. Of these certainly the latter and probably the former may be accepted without further question. But upon similar subtraction for second meals we find the area for internal cold the same as that for regular milk (actually -91 sq mm) and the area for external cold merely 428 sq mm less than the regular milk average. Apparently the exhibition of cold, after stimulation of the stomach by a previous meal, is unable to unlock the mechanism already set in action. It may be that this bears an important practical relation to the drinking of cold fluid during a meal where the unlocking of the mechanism set up by the meal would have a retarding effect upon the gastric action. At least the subject will bear further investigation. We may have struck a significant lead here.

FLUOROSCOPIC OBSERVATIONS

Immediately upon the ingestion of a hot milk meal the Magenblase distends laterally. This fact finds its indication in the slightly increased gastric width shown in Table I. We have not found a similar phenomenon with the exhibition of cold or even with the application of heat to the abdominal wall. In all these experiments the Magenblase, like the gastric tube, is reduced in width.

Heat, whether exhibited locally as a hot drink or indirectly through the abdominal wall, induces immediate activity. The failure of the stomach shadow to elongate or distend is quite noteworthy and stands in contrast even to the small change in dimensions seen after the ingestion of a milk meal at 70° F. Peristalsis starts immediately in the short narrow shadow. It at once extends from lower gastric tube to pylorus and may rapidly involve the entire stomach commencing just beneath the Magenblase. The waves are not massive like those of buttermilk. They rarely pinch off the entire gastric lumen. But they are of considerable amplitude and follow each other in rapid succession. They are short, frequent, vigorous waves whereas the buttermilk waves are long, massive, frequent and manifestly powerful. The heat activity lasts for about twenty minutes, scarcely becoming reduced with the progress of time, and is accompanied by rapid rhythmic passage the shadow of which is slight as in a regular milk meal not massive black and moniform as after buttermilk. At the expiration of twenty minutes after taking one of our small 5 ounce meals gastric activity subsides and the stomach enters the neutral phase. On the fluoroscopic screen the activity of the stomach after external application of heat is much more marked than that of the gastric response to a drink of hot milk.

When a cold milk meal is taken or a milk meal at 70° F is swallowed after application of cold to the abdominal wall the Magenblase distends up

wards, the cupola rising with it. This form of enlargement is in marked contrast the lateral distension of the Magenblase following a drink of hot milk. The elongation and lateral distension of gastric shadow are indistinguishable from those seen after one of our regular milk meals. Immediately peristalsis is usually set up though it may be delayed for a minute but it is not pronounced as after heat, so that one has great difficulty in distinguishing or demonstrating any difference from the ordinary gastric response to regular milk. The striking feature, however, induced by cold is the rapid reduction

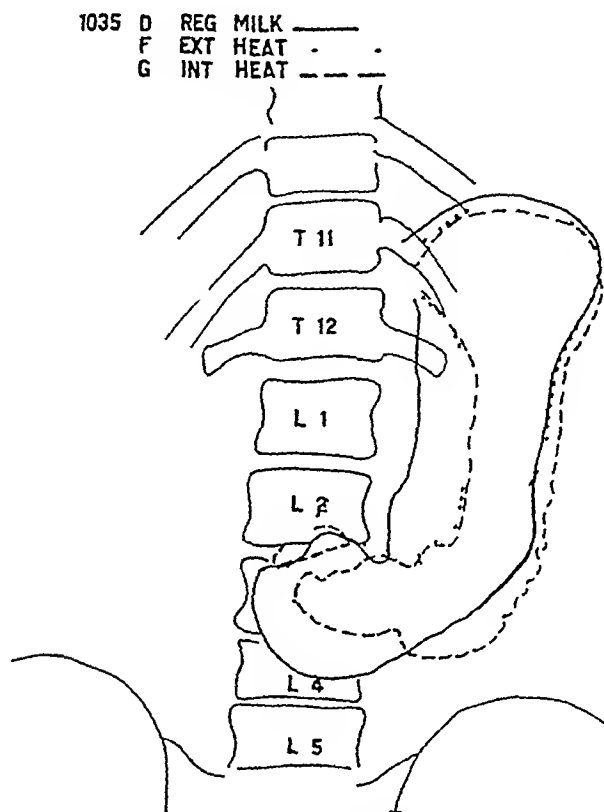


Fig 1—Reflex effect of heat upon stomach outline. The shadow is shorter, narrower and smaller than that following a regular milk meal at 70° F. Peristalsis is much more vigorous. Stimulation of the abdominal wall by heat has more pronounced effect upon gastric response than direct stimulation of the gastric mucosa.

of peristaltic activity so that, at the end of five minutes, the stomach is quite lethargic. This condition of diminished activity lasts until some twenty minutes after swallowing of the meal when the stomach enters its neutral phase.

Now when the stomach is in this neutral phase neither the nature of its contents, the character of the meal nor its temperature can be discerned.

Drawing together the results of all our experiments on heat and cold we would emphasize the fact that the effects of these stimuli upon gastric response are more marked when applied externally to the abdominal wall than when they are exhibited to the gastric mucosa itself. We are therefore justified in concluding that the response is reflex and not direct.

PICTORIAL REPRESENTATION OF RESULTS

We have adopted two methods of presenting our results pictorially. One of these, not adapted for use in journals, is the manufacture of moving picture studies from serial radiograms taken every twenty seconds. This method is quite satisfactory and enables us to study the serial radiograms without fatigue. It has already been described in a former communication.⁷ The other method is the superposition of tracings made from the radiograms of a trained stomach to show its response to various stimuli. In actual practice variations

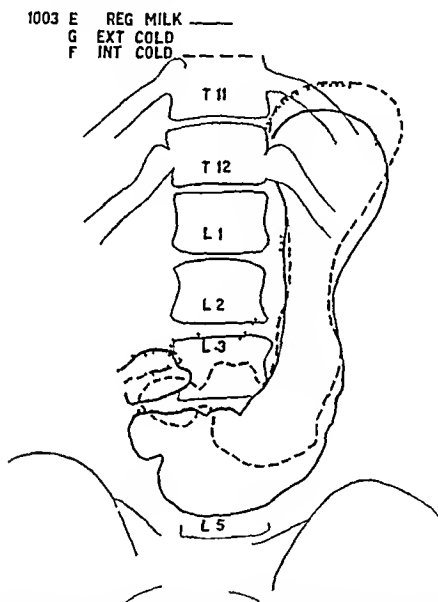


Fig. —Reflex effect of cold upon stomach outline. The shadow is narrower almost as long and little if any smaller than that following a regular milk meal at 70° F. Peristalsis is vigorous for five minutes and then becomes progressively less active than after the regular milk meal. Stimulation of the abdominal wall by cold is more effective in its action on gastric response than direct stimulation of the gastric mucosa.

in the stance of the subject may make it difficult to superpose shadows of the several vertebrae and the iliac crests quite exactly, but a compromise is readily found whereby the approximation is made very close. Figures 1 and 2 illustrate the application of this method to the presentation of results for heat and cold.

In figure 1 the relative dimensions are shown for milk at 70° F, milk at 140° F, and for milk at 70° F after the application of heat to the abdominal wall for 45 minutes. By a fortunate chance also, in this student, the relative activity is also shown. In figure 2 the responses to cold are similarly con-

trasted with the response to a regular milk meal. The upward distension of the Magenblase and raising of the cupola are well seen. The relative activity shown in these particular radiograms is not so characteristic as in figure 1 but the presentation of motility by static pictures is at best a hazard and the motility indicated by these pictures should be discounted. For adequate estimation of motility fluoroscopy is essential.

SUMMARY

1 In trained stabilized stomachs the effects of heat and of cold on the gastric reaction pattern can be demonstrated by the exhibition of a regular milk meal heated to 140° F or cooled to 32° F. They can be even better demonstrated by giving a regular milk meal at 70° F after applying a hot water bag or an ice pack to the abdominal wall during forty-five minutes preceding the feeding.

2 Inasmuch as the effect of the stimulus is more pronounced when applied indirectly through the abdominal wall than when directly applied to the gastric mucosa we conclude that the response is reflex in character.

3 Heat induces an intensely active stomach of small dimensions which is quite different in pattern from the active stomach induced by buttermilk. It enters the neutral phase after twenty minutes.

4 The application of external heat has no specific effect on the Magenblase but a hot drink brings about a broadening of the Magenblase.

5 Cold, whether applied directly or through the abdominal wall, has little or no effect upon total area of shadow. It induces a long narrow stomach outline with a Magenblase distended vertically, accompanied by rise of the left cupola. The stomach is quite active for five minutes and then becomes progressively lethargic. Twenty minutes after ingestion of the meal the stomach enters its neutral phase.

ABSTRACT

The effects of heat and cold upon gastric reaction pattern are reflex in their action because the resulting reaction pattern is more pronounced when the abdominal wall is stimulated than when the gastric mucosa is directly stimulated.

Heat results in a narrow, short, small stomach shadow with markedly active peristalsis during twenty minutes when, after a 5-ounce meal, the stomach enters its neutral phase.

Cold induces a narrow but not a short shadow. The Magenblase distends vertically. The shadow area is but little less than that induced by a regular meal. Peristalsis is active for five minutes and then the stomach becomes progressively lethargic until the neutral phase is entered twenty minutes after swallowing the meal.

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STUDIES IN THE ALIMENTARY TRACT OF MAN^{*}

V DISTURBANCES OF CENTRAL ORIGIN IN GASTRIC RESPONSES

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INTRODUCTION

IN ONE of our previous articles¹ we have called attention to the disturbances in gastric response brought about by impulses of central nervous origin. We were puzzled because the Sophomore stomach showed a reaction pattern differing from that exhibited by the same student as a Freshman even when the conditions of the experiment were deliberately duplicated.

The complexity of the gastric reaction pattern and the difficulty of separating out interferences of nervous origin are illustrated in our articles dealing with the responses to buttermilk² and to heat and cold.³ We found that, in experiments involving the use of milk or buttermilk or of heat whether as a hot drink or as a hot water bag applied to the abdominal wall, the second meal given after an interval of an hour was more effective than the first meal. This phenomenon we have called facilitation. But we have found no such result in experiments involving cold and we assume, as a working hypothesis, that cold is unable to change the mechanism already set in action.

It is apparent that these studies on the alimentary tract cannot be fully developed without a proper consideration of the effect of psychological influences, or perhaps one might better express it, the effect of stimuli originating in the central nervous system. It has been entirely proper to withhold this discussion so far in view of the difficulties encountered, but the training of medical and non-medical stomachs during the past five years has by now, given us a large amount of material from which selection can be made.

It is our experience that when a student first presents himself as a Freshman in Anatomy he is apt to exhibit symptoms of disquietude which are exaggerated when he comes to his roentgenoscopic examination. The symptoms are pallor, flushing, dryness of the mouth, difficulty in speech, a cold and clammy skin with perspiring palms. The physical movements are jerky and nervous. It is necessary to emphasize this description which is drawn from the records made by non-medically trained observers. It cannot be considered a biased statement but constitutes a simple recital of actual fact. However, it is not to be assumed that all students react in this manner. This condition is marked in some and entirely absent in a few, but most students exhibit some disquietude. Along with the outward manifestations we may expect others, more obscure, affecting the internal organs.

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Any tendency present in the student toward the manifestation of disgust is exaggerated at his first roentgenoscopic examination by the very newness and strangeness of the experience by its unexpected character and by the necessary accessories of the technique, the darkness, the close confinement, the odor of ozone, the hum of the motor, the spitting of the high tension, and the static discharges.

As the student becomes accustomed to the routine the symptoms diminish. His growing experience of medical training and his increasing knowledge of the principles of medical science develop his understanding, and, by the time he is a Sophomore, he rarely exhibits any outward indication of disgust.

Generally speaking comparison of the several records, fluoroscopic and radiographic, of each student shows a progressive reduction in dimensions of gastric shadow and an increasing gastric activity in reaction pattern as the student's status is raised through the Freshman to the Sophomore year. Nevertheless, a sudden unexpected stimulus produces an immediate effect which, despite the rapid recovery, points toward the condition characteristically found and more prolonged in duration in the Freshman.

THE INVESTIGATION OF EMOTIONAL DISTURBANCE OF GASTRIC PATTERNS

One of the first essentials in a policy looking towards stabilization of gastric responses is the avowed elimination of all "trials." Students at the beginning were quite naturally apprehensive of their exploitation for purposes other than that so clearly affirmed namely their own education in medicine and the mutual investigation of normal gastric behavior. It was difficult for them to realize that in a quarter of a century of roentgenological technique no serious and long sustained attempt has been made to analyse critically the gastric responses. The lapse of years during which we have faithfully lived up to our declared principle has allayed this fear. It has also taught us the worthlessness of such experiments as have been done by others purporting to show the modification of gastric response by emotions suddenly "evoked" by the subject himself as a result of his own thoughts or volition. These supposed reactions have been registered through tambours by lever on a smoked surface without any control of observation to learn what relation if any exists between the record and the details of gastric behavior. We have learned that the very fact that a subject knows he is to be the object of a "psychological" study produces such an effect upon gastric reaction pattern that the result of the particular stimulus used will be a distorted one. We realize also that any stimulus once deliberately used can never be employed again. Under such circumstances how can we plan experiments which shall be direct enough to have any definite value. We believe this can be done by taking advantage of incidents which are bound to happen in any long continued study. But in order to take proper advantage of them the investigation must be planned to provide for eventualities.

There are two classes of interference which call for attention. The one is momentary, the other sustained. The former comprises mental shocks the latter includes mental strain. In this article we propose simply to indicate possible lines of investigation.

THE RECORD OF MENTAL SHOCKS

A simple instance of this susceptibility can be demonstrated by an apparent blunder in technique. Student No 1027, at his third gastro intestinal examination, had already been observed at the fluoroscopic screen and now stood in the regular position, prepared for the 10-minute radiogram. At the moment of exposure the operator's thumb slipped off the timing switch and the initial noise made by the throwing in of the high tension was instantly interrupted. The operator recovered the switch immediately and the exposure was made with a delay of approximately one second. Suspecting that this radiogram would be a failure, the student was exhorted to remain steady in position and a second radiogram was taken after the lapse of one minute. The student was then released from this position and allowed to walk about the room. Five minutes later, a third radiogram was made of his stomach. Throughout this experience the patient was composed and absolutely unaware of any disturbing influence caused by the interrupted exposure during the taking of his first radiogram. Nevertheless, the result, on this first radiogram, showed clear indication of disturbance by modification of sites of pylorus and greater curvature and by absence of peristalsis. The positions of the various gastric features on the three successive radiograms are given in Table I.

TABLE I
PSYCHOLOGICAL INFLUENCE ON STOMACH POSITION

		TIME	CARDIA	PYLORUS	GREATER CURVATURE
1027	H	4 55 P M	MT12	ML3	LL4
	I	4 56	MT12	UL3	ML4
	J	5 00	MT12	dL2 3	dL3 4

The constancy in position of cardia is quite characteristic of our studies in general. The elevation of pylorus is unusual and is to be interpreted as evidence of recovery from a minor gastric collapse resulting from the registration by the student's stomach of a stimulus which made no impression upon his consciousness. The site of greater curvature shows the same response in more marked degree. The simple recital of these changes is not in itself convincing but they are accompanied by equally significant changes in gastric motility. In figure 1, radiogram H (4 55) shows evidence of very shallow peristalsis and the gastric tube is relatively broad. In the succeeding radiograms, I (4 56) and J (5 00), the gastric tube is narrower and peristalsis is apparent. Indeed in J the existence of a marked wave of peristalsis has deflected the greater curvature so that at one point it reaches the upper border of the fourth lumbar vertebra.

It might be objected that the foregoing observations may equally well be characteristic of any repeated radiography. This is not our experience. We have made several studies by serial radiography, and in no one is there any evidence of this phenomenon. Indeed, exaggerated gastric dimensions, usually known as hypotony, and diminished peristalsis are the two features which we find characteristic of the disquietude symptom-complex.

Repeated observations of such temporary evidence of disquietude as just noted in student 1027 are seen in fluoroscopic examinations when it is not always possible to make permanent records. On damp days when there is a greater static discharge from the supporting bar of the fluoroscopic screen to the student, we not infrequently see a sudden gastric disturbance which can be definitely associated with a static discharge.

Another student, No 1030 a Sophomore actually under observation on the fluoroscopic screen received a telegram of which he was immediately apprized. The regular peristaltic rhythm ceased instantly and the greater curvature of the stomach shadow dropped. He was then released to read his

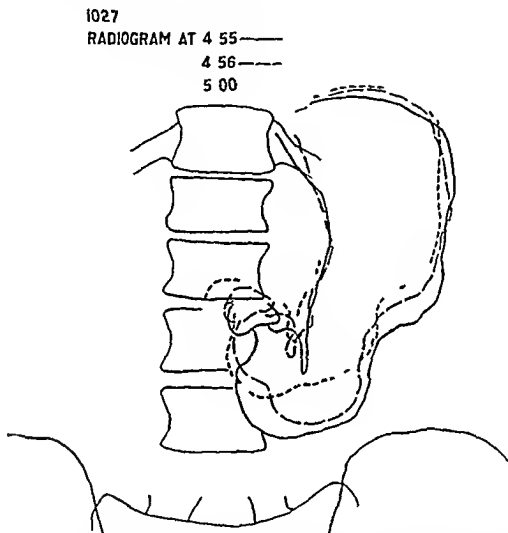


FIG. 1—Influence of momentary mental shock. Recovery of stomach from a sudden unexpected stimulus as shown in three successive radiograms at intervals respectively of one minute and four minutes.

telegram which proved to be a mere request requiring consideration. Twenty minutes later, after attending to the reply, the student was again examined at the screen. Peristalsis had reestablished itself but the greater curvature had not yet returned to its original position.

Student 1025, during his Sophomore examination was being suspected at the fluoroscopic screen when a sudden flashing occurred in the Coolidge tube resulting in an immediate break in the circuit. The consequent gastric collapse was apparent and lasted for somewhat less than an hour.

It is not necessary to provide a mental shock in order to produce temporary inhibition of peristalsis. We regard it as usual to find entire absence of peristalsis for one minute after recalling a student to the screen. At the expiration of this time waves again make their appearance.

THE RECORD OF MENTAL SHOCKS

A simple instance of this susceptibility can be demonstrated by an apparent blunder in technique. Student No 1027, at his third gastro-intestinal examination, had already been observed at the fluoroscopic screen and now stood in the regular position, prepared for the 10-minute radiogram. At the moment of exposure the operator's thumb slipped off the timing switch and the initial noise made by the throwing in of the high tension was instantly interrupted. The operator recovered the switch immediately and the exposure was made with a delay of approximately one second. Suspecting that this radiogram would be a failure, the student was exhorted to remain steady in position and a second radiogram was taken after the lapse of one minute. The student was then released from this position and allowed to walk about the room. Five minutes later, a third radiogram was made of his stomach. Throughout this experience the patient was composed and absolutely unaware of any disturbing influence caused by the interrupted exposure during the taking of his first radiogram. Nevertheless, the result, on this first radiogram, showed clear indication of disturbance by modification of sites of pylorus and greater curvature and by absence of peristalsis. The positions of the various gastric features on the three successive radiograms are given in Table I.

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punishment by the Faculty for dishonesty in an examination, the penalty for which is expulsion from the school with consequent termination of his medical career. Figure 2 shows superimposed tracings of radiograms taken at the time of his two gastro intestinal examinations. The stomach, upon the occasion of the first examination, is one peculiarly free from the usual Freshman collapse. At the second examination the stomach exhibits a general ptosis. It is quite common in our experience to find a lowering of greater curvature. A drop in pylorus more rarely occurs, an example being No. 1027 just described. A lowering of cardia in addition, is extremely rare. In this example, the cardia is lowered three vertebral units, the pylorus four and the greater curvature six. There is therefore a profound disturbance in stomach position. It might be thought that modification of respiratory position from one radiographic session to another might account for the lowering of cardia. Our experience is unequivocally contrary to such an explanation. Change of position by one unit should be considered negligible because of the technique of registration. Change of position by two units is more significant. Change of position by three units indicates considerable disturbance. In prolonged collapse such as this the picture of peristalsis need not be affected and, in deed, peristalsis was found moderately active in No. 324.

Another example of this kind is to be found in student No. 12 who was also awaiting the investigation of grave charges. He understood fully the possible effect of his mental situation upon his gastric response and was determined that there should be no visible effect. Our fluoroscopic study and the radiograms taken showed no dropping whatever of the greater curvature compared with the studies made when he was in normal mental condition. We however gave him both milk and buttermilk meals and found that even buttermilk failed to elicit any peristaltic activity. As the examination was made on the very day when the horrible situation first dawned on him we feel that inhibition of peristalsis was probably a direct effect.

THE EFFECT OF DEPRESSING MENTAL STRAIN

The first group of examples presented was one illustrating temporary mental shock, the second comprised instances of mental distress merging into apprehension. We shall now present an instance from the third category namely a student under prolonged strain it being understood that strain signifies here depressive strain not exhilaration. We do not expect, therefore, any disturbance of the peristaltic record. We shall look for an effect produced upon the gastric dimensions. The student in question is No. 327 a man, quiet, modest, and rather diffident, but a good worker and an earnest student. For contrast with his record we shall include that of No. 304, another student of very similar characteristics. Both these men exhibited the usual evidence of disquietude in their Freshman October examination, and the radiographic outline of their stomachs corresponded with these external manifestations. With increasing experience and adjustment to the conditions of student life both showed reduction of gastric shadow dimensions and increased vigor of peristalsis during their February gastro intestinal examinations. Figure 3 shows the reduction in gastric dimensions and increased vigor of peristalsis

A momentary dropping of the greater curvature is characteristically present if the student stumbles as he steps on to the insulated platform behind the screen when recalled for further fluoroscopic examination.

These interferences are never seen if the student is warned of some impending experience. For example, considerable static is sometimes developed in ourselves as a result of the heavy insulation to which we are all subjected. The student is told of the possibility of sparks from his body to the grounded screen. When so warned there is no stoppage of peristalsis or

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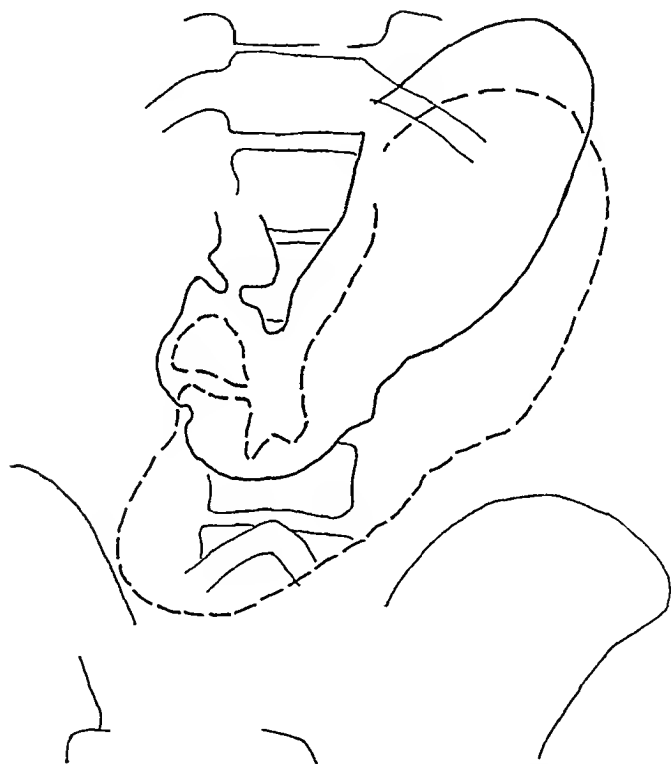


Fig. 2.—Influence of acute mental distress. The magnitude of the effect is shown by ptosis of pylorus and of the even rarer ptosis of cardia in addition to that of greater curvature.

modification of tone, both of which may occur with the first sparking if he is not warned.

In instances of minor effect the condition known as hypotony is induced but no interference is seen in peristalsis. When the effect is more exaggerated, peristalsis diminishes or is inhibited. In our experience peristalsis invariably returns before the dimensions of the stomach resume normality.

THE EFFECT OF MENTAL DISTRESS

Student No. 324 was examined as a Freshman in October 1925 and again in February 1926. At the time of the February examination he was awaiting

score of times. Shall we brush aside the obvious and search for an abstruse origin, or shall we reject the evidence because the abnormal response was not planned in advance? Surely we have said enough to demonstrate that interference planned ahead would be most unlikely to take the course predicted. We have, in the smooth muscle of the alimentary tract, a sensitive indicator of emotion which is not only wholly free from voluntary control but is also incapable of being dragged over the threshold of consciousness.

In our records are instances of the interference in gastric response by fatigue in its various stages and by the onset of sudden illness. These are still more difficult to analyse. They will be reserved to swell the growing mass of evidence on behavior patterns which slowly accumulates as the years pass and the conduct and comprehensiveness of our investigations grow ever more complete.

SUMMARY

1 The differences found between the gastric reaction patterns of Freshmen students examined for the first time and those of well trained and cooperative Sophomore students point to the presence of a disturbing factor of central nervous origin which is being minimized as the student progresses in his medical curriculum.

2 The effect of this factor is two fold, namely in a relaxation of the length phase of gastric smooth muscle known as hypotony and in inhibition of peristalsis.

3 Both effects may be present or one only occur, depending upon the various complicating and modifying conditions encountered.

4 Such central disturbances may be divided into three categories:

A The purely temporary effect of mental shock.

B The more prolonged results of mental distress.

C The long sustained modification of behavior pattern by mental strain.

5 In mental shock there is an inhibition of peristalsis of evanescent character and a "lowering of gastric tone" which is of greater duration, depending upon the character of the stimulus.

6 In mental distress there may be lowering of tone or inhibition of peristalsis but if the latter be present it is of such fixity that it cannot be removed by the stimulus of buttermilk (or lactic acid).

7 In mental strain we do not expect inhibition of peristalsis but find a quite definite change in length phase (lowering of tone). It is this phenomenon which makes itself apparent in the October Freshman.

8 The very nature of these interferences in behavior pattern makes it certain that they would not be encountered in their simple forms if the experiments were planned ahead.

ABSTRACT

Interferences of central nervous origin can and do make their presence felt in gastric behavior patterns. The analysis of these interferences can be

most efficiently carried out from a study of such instances as occur in a large clinic of carefully conducted investigation. The fore-knowledge of an impending stimulus is enough in itself to distort or even to break down the experiment completely. We have studied the effect of mental shock, mental distress and mental strain, and here present our analysis of the data obtained.

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LABORATORY METHODS

A STUDY OF THE NEW FOLIN MICROMETHOD FOR THE DETERMINATION OF BLOOD SUGAR*

By S. L. LEIBOFF, A. M. and DOROTHY KOPPEL, B. S., NEW YORK, N. Y.

RECENTLY Folin¹ developed a method for the determination of sugar in 0.1 cc of blood. In this method the sugar is oxidized with alkaline potassium ferricyanide as in the method of Hagedorn and Jensen. In the Hagedorn-Jensen method the amount of reduced ferricyanide is determined by titrating with an iodine solution while in the Folin method it is determined colorimetrically as Prussian blue by the addition of a ferric salt.

We felt that this method, if proved of sufficient accuracy, should find a place among laboratory procedures. It occasionally happens that one has difficulty in obtaining blood from a vein. Also in the treatment of diabetic patients, where a blood sugar determination is required often it should be of particular value.

From the beginning of our investigation of this method we have encountered an obstacle in that the gum arabic failed to hold the Prussian blue in dispersion for a sufficient length of time. This was particularly the case with blood filtrates containing abnormal amounts of sugar. We have tried various grades of gum arabic, and while some were better than others, we could not rely upon them. We have also tried other protective colloids without much success.

A good protective agent for Prussian blue is oxalic acid. This acid has been used for some time as a peptizing agent for Prussian blue in the preparation of colloids. We have found the alkali salts of oxalic acid to be particularly effective. We have used a 1 per cent solution of potassium oxalate which was added after the addition of the ferric salt (no gum arabic was present in the ferric salt). A number of tubes were set up containing the same amount of sugar but with varying amounts of oxalate. The first appearance of a precipitate was noted by means of a hand lens. As is shown in Table I, the oxalate is a good protective agent for the Prussian blue.

TABLE I
LENGTH OF TIME THAT PRUSSIAN BLUE IS PROTECTED AGAINST PRECIPITATION BY POTASSIUM OXALATE

TUBE	POTASSIUM OXALATE (1 PER CENT)	TIME OF PROTECTION
1	0.1 cc	26 minutes
2	0.2 cc	33 "
3	0.3 cc	47 "
4	0.5 cc	60 "
5	0.7 cc	78 "
6	1.0 cc	No protection after 2 hours
7	None	8 minutes

From the Biochemical Laboratory of the Lebanon Hospital, New York.

However, we soon had to abandon the use of oxalate, since we found that it exerts a bleaching action on the Prussian blue, thus giving low and unreliable sugar values. We then turned our attention back to the gum arabic, and after some experimentation we devised a simple technic for the preparation of a purified gum arabic which gave good protection to the Prussian blue for a sufficiently long time.

PURIFICATION OF GUM ARABIC

Eighty grams of crude gum arabic are ground to a powder and introduced into a liter Erlenmeyer flask to which is added about 600 c c of 10 per cent acetic acid. Solution is brought about by occasional shaking. No heat is applied. When most of the gum is dissolved, which takes a few hours, it is filtered through a Buchner funnel with suction. It is best to filter not more than 200 c c at a time and to change the filter paper before adding more gum arabic solution, as otherwise filtration becomes very difficult. The filtrate is then transferred to a large beaker, and two volumes of 95 per cent ethyl alcohol are added, a snow white flocculent precipitate is formed at once. This is filtered on a Buchner funnel and washed with 70 per cent alcohol until the washing is free from acid. The gum is then placed in a shallow porcelain dish and heated on a water-bath until dry.

A 10 per cent solution is prepared by heating the gum arabic in water on the water-bath until dissolved, and filtered while hot through filter paper in an ordinary funnel, no suction being required. The gum dissolves very readily and filters easily.

We do not add the gum arabic to the ferric sulphate solution but keep it in a separate bottle. The ferric sulphate-phosphoric acid mixture is prepared according to Fohn but without the gum arabic. In the test for sugar we add one c c of the 10 per cent gum arabic solution immediately before adding the ferric sulphate. The gum is kept separate because it prevents the precipitation of Prussian blue for a much longer time than when the two are kept in one bottle. With bloods containing up to 200 mg of sugar per 100 c c of blood, at least one hour will elapse before any visible precipitate can be detected.

The amount of protection given to the Prussian blue by the purified gum arabic depends upon the amount of sugar present. The less the amount of sugar the greater the protection. This is shown in Table II.

TABLE II
PROTECTION OF PRUSSIAN BLUE BY THE PURIFIED GUM ARABIC

TUBE	MG SUGAR IN 100 CC OF BLOOD	TIME OF PROTECTION
1	50	No protection after 2 hours
2	100	70 minutes
3	150	61 "
4	200	58 "

The most accurate results by this method are obtained when the unknown and the standard contain about the same amount of sugar. This is due, to a great extent, to the variation in the amount of ferri-cyanide present in the

different tubes, producing a greenish tint of varying intensity, thus making the matching of colors rather difficult.

This difficulty we overcame to a great extent by using three samples of the unknown sugar solution containing varying amounts of sugar, as follows:

Tube 1 contains 4 cc of filtrate

Tube 2 contains 3 cc of filtrate + 1 cc of water

Tube 3 contains 2 cc of filtrate + 2 cc of water

For the colorimetric comparison that tube is chosen the color of which most resembles that of the standard.

The calculation is as follows:

$$\text{Tube 1 } \frac{20}{R} \times 100 = \text{mg sugar per 100 cc of blood}$$

$$\text{Tube 2 } \frac{20}{R} \times 100 = \text{mg sugar per 100 cc of blood}$$

$$\text{Tube 3 } \frac{20}{R} \times 100 = \text{mg sugar per 100 cc of blood}$$

The use of three tubes has another advantage in that it enables us to determine up to 450 mg of sugar without having to repeat the determination in bloods containing large amounts of sugar as 1 cc of the ferricyanide will take care of only 225 mg of sugar.

We have checked this method against that of Folin Wu³ on a large number of bloods. We used the Folin Wu filtrates which we diluted 1 to 10 thus making a final dilution of 1 to 100. The results obtained were invariably lower than those obtained by the method of Folin and Wu, thus confirming the findings of Folin. Table III gives the figures for a few of our determinations.

TABLE III

METHOD OF FOLIN WU	METHOD OF FOLIN
130	119
94	91
109	104
119	108
123	119
188	162
99	90
240	214
125	115
117	111
124	117
237	216
262	230
81	80
85	83
102	97
118	112
90	90
88	85
173	166

We then did a number of determinations to see whether good checks could be obtained by this method on samples containing the same amount of sugar. These determinations were done on triplicate samples and checked against

tuplicate samples by the Folin-Wu method Table IV shows the checks by the Folin method were not quite so good as those obtained by the Folin-Wu method

TABLE IV

FOLIN WU METHOD			FOLIN METHOD		
87	86	86	84	79	81
98	97	96	96	90	92
137	140	138	133	134	129
216	209	218	202	208	209
255	261	252	238	247	230
81	81	80	73	79	75
92	94	91	85	88	87
106	104	108	98	103	100

Recently Folin¹ has published a supplementary note on his new sugar method in which he recommends the use of Ghatti gum instead of the gum arabic We have not tried Ghatti gum, for we obtained satisfactory results with the purified gum arabic Although this necessitates the use of two separate solutions we see no particular disadvantage in it

We prepare our standard fresh daily by diluting the Folin-Wu standard 1 to 10 with water and we have had no difficulty from this quarter

SUMMARY

1 The new blood sugar method of Folin was tested and checked against the method of Folin and Wu While this method is not quite so accurate as the Folin-Wu method and is not meant by Folin as a substitute for the Folin-Wu method, we believe it deserves a place among laboratory procedures

2 A technique is described for purifying gum arabic which is used as a protective agent for the Prussian blue

3 The use of three tubes in the test is recommended thus allowing one to approximate very closely the color of the standard, thereby obtaining more accurate results By doing this no repetition is necessary in cases with a high blood sugar content

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SOME MODIFICATIONS IN THE DETERMINATION OF NONPROTEIN NITROGEN IN BLOOD*

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ALL the methods used for the determination of nonprotein nitrogen in blood are based upon the same principle that of the original Kjeldahl method of digestion with sulphuric acid by means of which the nitrogen is converted into ammonium sulphate. In the Kjeldahl method the wormed ammonia is liberated into an acid medium by means of distillation and is then titrated with a base of known normality, a very cumbersome procedure. Myers¹ substituted aeration for distillation. Folin and Denis substituted nesslerization of the ammonia for titration thus making it a colorimetric procedure. Later Folin and Wu² have devised a procedure whereby the distillation or aeration was entirely eliminated, the Nessler solution being added directly to the ammonium sulphate. However in this method a great deal of attention must be paid to the proper size of the flame used for digestion and also to the proper length of time of digestion or the phosphoric acid present in the digestion mixture will precipitate silica from the glass of the digestion tube and produce cloudy solutions with the Nessler reagent. It is also necessary to add water at the proper time before the mixture has cooled sufficiently. Failure to observe any of these conditions will make the determination useless.

These difficulties were finally eliminated entirely by the ingenious procedure of Koch and McMeekin³ who dispensed with the acid digestion mixture of Folin and Wu and introduced the use of sulphuric acid and hydrogen peroxide as the sole digesting agent. The use of hydrogen peroxide was first suggested by Myers. Koch and McMeekin also used a modified Nessler solution in which no mercurous salts are present.

This method has been used in this laboratory for the last two years with very good results.

While this method is quite ideal there are still two disadvantages present: (a) bumping of the solution during digestion thus being exposed to possible loss of nitrogen and (b) the hydrogen peroxide contains some nitrogen though the amount of nitrogen present in a few drops of 30 per cent hydrogen peroxide was found to be very small indeed and for practical purposes may be disregarded. The purpose of this paper is to introduce measures for overcoming these difficulties.

The greatest source of trouble is the bumping. Koch and McMeekin suggested that the digestion may also be done on the sand bath. However, the sand bath does not help much when test tubes are used. But by substituting flat bottom flasks for the test tubes this difficulty is completely overcome. We have used 50 c.c. pyrex volumetric flasks. No glass beads are necessary nor

*From the Biochemical Laboratory of the Lebanon Hospital, New York.
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desirable to prevent bumping. The boiling takes considerably longer but proceeds very smoothly and digestion is complete. The greater length of time is no objection since a great many determinations can be done at the same time. No watching is necessary so that other work may be done while the flasks are being digested.

The procedure is essentially that of Koch and McMeekin. 5 c c of Folin-Wu filtrate are introduced into the flask to which is added 1 c c of 50 per cent sulphuric acid and allowed to boil on the sand-bath until SO_3 fumes fill the tube, boiling is continued for five minutes longer. The flask is then removed from the sand-bath and allowed to cool for two minutes. Three drops of 30 per cent hydrogen peroxide are added and the flask is again placed on the sand-bath and boiling continued for five minutes after the SO_3 fumes have begun to come off. The flask is then allowed to cool to room temperature and is filled about two-thirds with distilled water. While rotating the flask 12 c c of modified Nessler solution are added, and water to the 50 c c mark. A clean stopper is inserted in the flask and the contents are well mixed and compared in the colorimeter against a special standard solution.

The graduation mark being on the neck of the flask has the advantage over the tube in allowing for more accurate dilution.

The small amount of nitrogen which is present in the hydrogen peroxide is taken care of by using a special nonprotein nitrogen standard which is treated with the same amount of hydrogen peroxide as in the blood filtrate. The standard is prepared as follows:

PREPARATION OF STANDARD

Weigh out 0.283 gm of pure ammonium sulphate (chemically pure ammonium sulphate recrystallized twice and dried at 100°C to a constant weight) and dissolve in water in a small beaker and wash it down into a liter volumetric flask, washing down the beaker with successive changes of water until the flask contains about 600 c c of water.

Into a 200 c c pyrex beaker or Erlenmeyer flask place 600 drops of 30 per cent hydrogen peroxide (use the same dropping bottle containing peroxide which is being used in the test, in order to have the drops of the same size). Add 25 c c of concentrated sulphuric acid and heat on the sand bath until SO_3 fumes are given off, and continue heating for ten minutes longer. When cool, add this to the volumetric flask, washing it down with a few portions of water. Add to the flask containing the ammonium sulphate, slowly, with frequent shaking, 175 c c of concentrated sulphuric acid. When cool, add water to the liter mark. Let cool again and add water to the mark, mix well and place in a well stoppered bottle.

This standard not only compensates for the nitrogen present in the hydrogen peroxide, but it will keep almost indefinitely.

This standard contains 0.3 mg nitrogen in 5 c c. For use, 5 c c of the standard are placed in a 100 c c volumetric flask about two-thirds filled with water and while rotating the flask, 25 c c of the modified Nessler reagent is added and diluted with water to the mark.

The calculation is the same as in the method of Folin and Wu. When the standard is set at 20 mm then

$$\frac{20}{R} \times 30 = m_{\text{NPN}} \text{ per 100 cc of blood}$$

A large number of determinations were performed by this procedure and were checked by the method of Koch and McMeekin using test tubes for the digestion. Very close checks were obtained. By using the flasks instead of the test tubes and by heating on the sand bath, closer agreements were obtained on duplicate samples. A few of the figures on duplicate samples are given in Table I.

TABLE I

DIGESTED IN TEST TUBES OVER MICRO BURNER		DIGESTED IN FLASKS ON SAND-BATH	
31.5	31.1	32.0	31.8
28.2	29.3	29.3	29.5
39.0	38.3	39.5	39.2
64.5	69.6	70.5	71.6
138.7	143	145.0	146.8
33.7	33.0	31.6	31.5
49.0	47.8	48.9	49.5
29.4	28.7	30.2	30.4

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POLLEN SUSPENSIONS A PRELIMINARY REPORT

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POLLENS are recognized as a causative factor in hay fever, either alone or in conjunction with the special constitutional tendency of certain persons.

Blackley,¹ in 1865 established this causal relation. Dunbar² in 1903 concluded that the protein constituent of the pollen grain was the active excitant. The present day method of treating hay fever was basically established by Noon and Freeman³ in 1911. It consists in hyposensitizing the patient with an extract of the offending pollen, the extract being a solution of the pollen protein.

A number of methods have been used for the preparation of protein from pollen. Noon and Freeman³ used distilled water, alternately freezing and thawing the mixture, filtering and boiling in sealed containers. Clowes⁴ used acetone for defatting, then extracted with distilled water. Lowdermilk⁵ used physiologic salt solution instead of distilled water. Clock⁶ introduced a solvent consisting of 33 $\frac{1}{3}$ per cent of saturated sodium chloride and 66 $\frac{2}{3}$ per cent of glycerol. Koessler⁷ used 8 $\frac{1}{2}$ per cent of sodium chloride as a solvent, later diluting with 10 volumes of distilled water, making the final extract contain 0.85 per cent of sodium chloride. Goodale⁸ employed 13 to 15 per cent by volume of alcohol in distilled water. Walker⁹ adopted a 12 per cent alcoholic saline solution. Rackemann¹⁰ employed physiologic saline solution rendered slightly alkaline with 1 per cent normal sodium hydroxide. Coca¹¹ employed an alkaline extracting medium buffered with sodium bicarbonate. Bernton¹² modified Coca's method by adding two parts of glycerol to one part of Coca's fluid. Stier¹³ by alternations in the character of the extracting fluid selected one containing 46 per cent glycerine, 7 per cent sodium chloride and 47 per cent distilled water as a superior solvent as measured by skin reactions.

An acetone insoluble fraction redissolved in saline solution was tried as a method of refinement. This fraction represented from 10 to 20 per cent of the skin reacting substance and therefore the method did not approach complete extraction.

I attempted to extract ragweed and timothy pollen exhaustively by repeated extractions with distilled water. After extracting each lot five times, the dried residue was still capable of producing positive skin reactions. Pollens apparently, are very reluctant to complete extraction of the exciting principle.

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The resume given shows a diligent search for a selective solvent for pollens, and the proteins in particular. The efforts have been fundamentally, in the direction of purification and refinement.

Piness, Miller and Alles¹⁴ using foods and animal emanations reported that the most effective solvent in extracting the natural proteins *without* denaturation for obtaining skin reacting substances is salt solution up to 10 per cent.

Alles and Lamson¹ state that instability, low concentration of active substance, and the presence of excessive amounts of substances added during extraction, present objections to the several methods proposed for extracting pollens.

Preparations of pollens or pollen extracts should be sterile, nonirritating, potent, stable, easy to use and adaptable in dosage.

Recognizing the role of pollen as a factor in causing hay fever it seemed logical to take into account the fact that pollen in its transit from plant to patient, is subject only to the climatic conditions of temperature and moisture and that it reaches the patient in a natural or crude state.

The inference is open here that attempts at purification and fractionation of pollen may result in denaturation of the active principle and that a better way to manage the hay fever problem would be to inject the pollen itself.

With this thought in mind work was started on the preparation of pollen suspensions to be used in the same manner as bacterial vaccines. Ramsdell¹⁵ employing pollen suspensions and pollen extracts demonstrated evidence of hypersensitiveness in rabbits and guinea pigs by complement fixation, precipitation, skin reaction, anaphylaxis and passive transfer of sensitization. While Ramsdell made no comment on the relative merit of pollen suspensions and pollen extracts in the production of antibodies the inference is permissible that they are at least equal in this respect and that sterile pollen suspensions would be suitable agents in the specific diagnosis and treatment of hay fever.

The writer in association with Masucci, Roos and McAlpine¹ reported on the use of phenols in extracting pollens employing pure trieresol and fused phenol. Abel and Geiling¹⁶ used 90 per cent phenol as a selective solvent in the purification of insulin. We summarized our investigation in regard to phenols by saying: pure phenol does not harm timothy pollen; it extracts a skin reacting substance, it removes an inert substance and affords a means of sterilizing whole pollen suspensions.

Having found a method of sterilizing whole pollen suspensions the next step was a study of absorption. It was realized that the size of the pollen grain and its cellulose structure had to be considered when injecting whole pollen suspensions. To facilitate absorption the pollen grains are reduced to about the size of bacteria. The sterile pollen suspensions were injected intradermally, subcutaneously and intraperitoneally into laboratory animals. Graduated suspensions of 1:100,000 to a pollen residue just thin enough to pass through a needle were used. All suspensions to 1:1000 were perfectly absorbed and caused no local disturbances. The massive pollen residue injected into the skin subcutaneously acted as a sterile foreign body with the forma-

tion of small sterile pustules. This same mass caused no untoward symptoms when placed in the peritoneal cavity. Animals killed after five days revealed no pathologic condition, nor any evidence of the material remaining within the peritoneum, indicating complete absorption.

Pollen suspensions were then used for testing known sensitive patients, by the intradermal method, in dilutions of 1 100,000, 1 20,000, 1 5,000, 1 2,000, and 1 1,000.

The tested patients were carefully observed. Local reactions, typical in character, appeared in fifteen minutes and passed through the usual stages of subsidence. The heavier suspensions showed a darkened spot at the deposit site, which entirely disappeared in four days, and there were no residual nodules or subjective symptoms of any kind.

A ragweed pollen suspension was substituted for an extract in the course of treatment in one patient, and it was found that he tolerated the suspension perfectly, with complete absorption, and that the interval between injections could be extended much beyond that usual in his case, with a better clinical control of symptoms than with the use of an extract.

Further clinical trial of pollen suspensions in the treatment of hay fever is in progress. The only comment permissible, at this time, is that the suspensions are well tolerated, are no more painful than clear extracts, and are rapidly and completely absorbed. Periodic itching at the injection site has been noted, but this, also, has been observed with the use of extracts. A subsequent report on the therapeutic value of pollen suspension will be made when sufficient data are available.

The suspensions are prepared by maceration to disrupt the pollen grains, the pollen mass is sterilized with phenol, and suspended in buffered salt solution, containing 0.5 per cent phenol as a preservative.

DISCUSSION

The plan to employ suspensions of whole pollen, prepared as described, in the treatment of hay fever is based on the idea of using pollens in their entirety, and as near as possible to their natural state.

It has been shown that pollen suspensions can be sterilized, they are non-irritating, potent, easy to use, adaptable in dosage, and can be supplied with a minimum of denaturation.

They are readily absorbed and cause no untoward signs at the injection site, nor has a limited clinical use developed any objections.

They are presented for investigation, hoping other workers will become interested and try them, with the larger hope that they may prove more effective in the treatment of hay fever.

NOTE. Pollen Suspensions were used for treatment during the past season in both the early and late types of hay fever. Ten new cases, previously untreated, gave an average percentage relief of 77 per cent, ranging from 50 per cent to 100 per cent. Seven cases previously treated with pollen extracts gave an average percentage relief of 90 per cent, ranging from 80 per cent to 100 per cent.

A 1 1000 suspension was used, the dose beginning at 1/10 c.c. progressing, in a few patients, to 2 c.c.

Constitutional reactions were noted in two patients, coming on much slower than with extracts, from 6 to 12 hours after infection, in one instance following a purposefully large first dose of $\frac{1}{4}$ cc in a previously treated patient, and the other, when a dose of $1\frac{1}{4}$ cc was reached

The average benefit for the seventeen cases was 82 per cent which compares very favorably with results obtained with pollen extracts

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THE SPECTROPHOTOMETRIC DETERMINATION OF HEMATOPORPHYRIN IN URINE*

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OUR interest in the possibility of detecting and of measuring hematoporphyrin in urine was stimulated by the fact that various types of dermatosis are found clinically in which the presence of a photodynamic substance may be postulated (or demonstrated in certain lesions) in order to explain the effects produced by exposure to light. We have presented elsewhere[§] a clinical study of a patient with eczema solare. We are presenting here the results obtained by apparatus and methods used for the detection of hematoporphyrin in urine.

Spectroscopy and spectrophotometry furnish accurate and rapid methods for the detection of small quantities of substances which, when in solution, give characteristic absorption zones or bands. The presence of very small quantities of any given substance in solution, however, may not be detectable with certainty by the use of the spectroscope for the reason that the percentage transmission of light throughout the spectrum may be so high as to obscure the presence of a low percentage of absorption of light in the region or regions characteristic of the dissolved material. For this reason, as well as for other reasons which might be given, spectrophotometry is often the method of choice, for it is possible to measure accurately the percentage of transmission (or absorption) of light for any given wave-length, thus enabling the observer to plot a curve showing the relationship existing between wave-lengths and percentages of transmission (absorption) of light. The quantity of the substance in solution may be estimated from an application of the laws of Lambert and of Beer. Since, then, the extinction coefficients e , are proportional to the negative logarithms of the unabsorbed light, I , and since the extinction coefficients are proportional to the concentrations, C , it follows that

$$\frac{e_1}{e_2} = \frac{C_1}{C_2} = \frac{-\log I_1}{-\log I_2} \quad (1)$$

If, therefore, it is possible to make a solution of hematoporphyrin which gives one or more characteristic absorption zones, the presence of hemato-

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§Goeckerman W. H., Osterberg A. E. and Sheard Charles. Eczema solare in a patient with hematoporphyrinuria. Arch. Dermat. & Syph. 10: 501-505, 1929.

porphyrin can be ascertained and the amount can be determined from an application of the equation (1) to the spectrophotometric data. We are recording in this paper a method which gives a characteristic absorption zone for hematoporphyrin in urine thereby permitting spectrophotometric determinations of the amount of hematoporphyrin present in terms of cubic centimeters of blood for each 1000 c.c. of urine.

THE DIRECT READING SPECTROPHOTOMETER

We have used in these investigations the direct reading spectrophotometer made by Kenuffel* and Esser and referred to in the literature as a "color analyzer" (Figs. 1 and 2). The instrument consists essentially of a lamp house carrying two blocks of magnesium carbonate (cut from the same cake) which are placed at the rear. These blocks serve as sources of light for transmission through the receptacles containing the liquids to be examined. The beams of light reflected by the blocks after transmission through two suitable openings in the front of the lamp house enter the two receptacles containing the solution and the solvent respectively. These tubes are placed in the

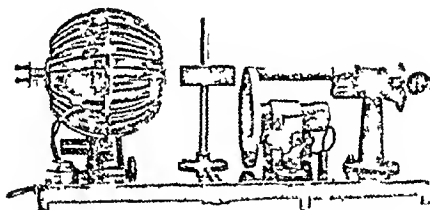


Fig. 1—The spectrophotometer or color analyzer

proper positions before the entrance slit of the spectrometer by an adjustment on the vertical supporting rod. The spectrometer proper does not differ in fundamental principles from the ordinary constant deviation type of instrument except for the addition of a biprism which is placed in front of the telescope lens system and an observing (exit) slit in the eyepiece. Throughout the series of observations which are to be reported in this paper the entrance and exit slits were kept at constant or fixed values following the initial adjustment of the entrance slit to give 100 per cent transmission (from the magnesium carbonate blocks) throughout the whole of the spectrum. A balance in the intensities of light transmitted by the standard cell and by the solutions under examination was made by means of a sector photometer, the intensity of the light transmitted by the standard cell only being varied at any given wavelength. The standard cell was filled with a mixture of alcohol and acetone (the solvent used); the other tube contained the solution to be examined spectrophotometrically.

The method of getting the data shown in Figs 3, 4 and 5 is as follows White light is passed through the two containers placed in front of the housing which carries the rotating sectors and is admitted to the spectrometer The spectrometer is set at any desired wave-length by means of a calibrated wheel For example, with a setting of 590 millimicrons (approximately the wave-length of sodium yellow of the spectrum), the observer, on looking through the ocular exit slit, sees two semicircular colored areas in juxtaposition, with the dividing line horizontal Both halves of the arch will have the same hue (yellow) but not necessarily the same brightness or saturation value

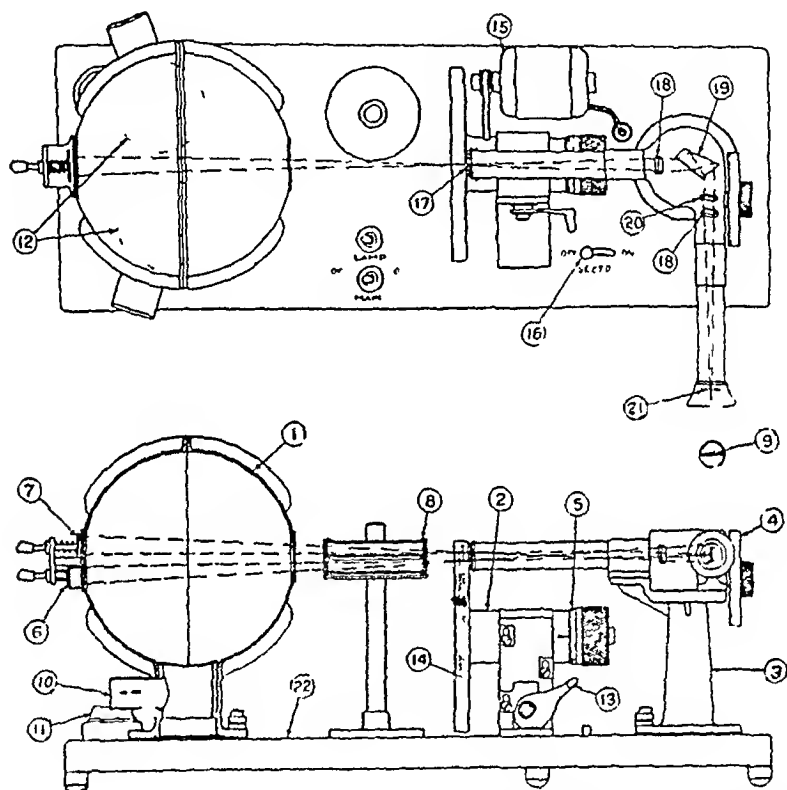


Fig. 2—Cross sections of working parts of the spectrophotometer of which the following are noted 1, spherical light house 2, photometer 3, spectrometer 4, wave-length scale 5, photometric scale 6, holder for standard sample 7, holder for reflection samples 9, field of view through the eye slit 11, sector disks 17, entrance slit 19, dispersion prism and 20, bismuth

The adjustment for the equality of brightness, or a match is then made by varying the size of the sector opening in the rotating sectors placed in front of the spectrometer The percentage transmission of the solution as compared to the percentage transmission of the solvent in the so called standard cell is read off directly from the calibrated drumhead This drumhead is connected mechanically to the sectioned disks in such a manner as to permit rapid turning of it by hand, thus providing a quick way of varying the relative proportions of open sector and closed sector areas In determining the percentage transmissions for any given wave-length it has been our custom to allow a few

moments for the adjustment of the eye to the spectral hue under observation and to record as the final reading for each wave length taken (ordinarily by steps of 10 millimicrons) the average of five determinations which do not vary more than two to three points respectively. After the measurement for equality of brightness for any given wave length has been made the procedure as outlined is repeated for as many determinations and for as many spectral regions as are deemed necessary.

SPECTROPHOTOMETRIC DETERMINATIONS OF HEMATOPORPHYRIN ADDED TO NORMAL URINE AND OF ALCOHOLIC SOLUTIONS OF HEMATOPORPHYRIN PRECIPITATED FROM URINE

The standard cell and the tubes containing solutions which were used in obtaining the data given in Fig 3 were 5 cm in length. Curve 2 is for normal urine, Curve 3 is for urine to which a trace of hematoporphyrin had been added, and Curve 4 shows the spectrophotometric data in a case in which

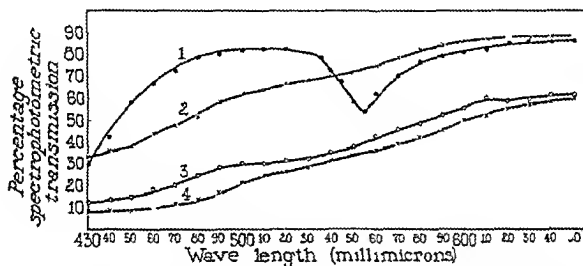


Fig 3—Spectrophotometric curves of transmission. Curve 1 acidified alcoholic solution of hematoporphyrin (5 cm tube) showing characteristic absorption zone with maximal absorption at 555 millimicrons. Curve 2 normal urine (5 cm tube). Curve 3 urine to which a trace of hematoporphyrin has been added (1 cm tube). Curve 4 a case in which hematoporphyrin in the urine was suspected.

hematoporphyrin in the urine was suspected. Inspection of these three curves does not show characteristic absorption bands or zones and further indicates that the shapes of the curves (their slopes at any given wave length) are similar. Curve 1, obtained with an alcoholic solution of hematoporphyrin, shows a definite absorption zone in the yellow green portion of the spectra, with the maximal point of absorption at 555 millimicrons.

Curve 1 of Fig 4 is the spectrophotometric curve given by an acid alcoholic solution of hematoporphyrin recovered from 1000 cc of urine. The quantity of hematoporphyrin represents an amount prepared from 0.75 cc of normal blood by treatment with concentrated sulphuric acid. The recovery was made by adding 50 cc of glacial acetic acid to 1000 cc of normal urine in which the added hematoporphyrin had been dissolved. The acid solution was allowed to stand twenty four hours. The precipitate was centrifuged and redissolved in 25 cc of alcohol with the aid of a small amount of dilute hydrochloric acid. Although the curve indicates clearly the presence of tur

bidity, since the maximal reading which occurs at 650 millimicrons is only 40 per cent, it definitely shows the presence of an absorption zone with a corresponding maximal absorption at 555 millimicrons. This same absorption zone with a corresponding maximal absorption at 555 millimicrons is shown in Curve 2, obtained from an acid alcoholic solution each cubic centimeter of which contained an amount of hematoporphyrin representing 0.00125 cc of blood. (In the original alcoholic solution, each cubic centimeter represented the hematoporphyrin from 0.005 cc of blood. Curve 2, therefore, was obtained with a dilution of $\frac{1}{4}$.) Curves 4 and 5 of Fig. 4 show the presence of hematoporphyrin in the urine of two persons tested. Curve 3 was obtained with an amount of hematoporphyrin equivalent to 0.25 cc of blood added to 1000 cc of urine, the recovery being made by precipitation with acetic acid and re solution in acid alcohol.

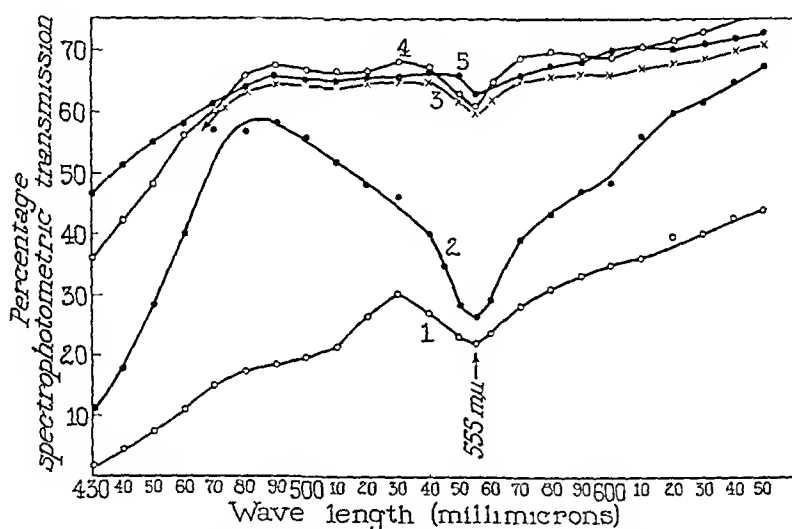


Fig. 4—Spectrophotometric curves of transmission. Curve 1 acid alcoholic solution of hematoporphyrin recovered from 1000 cc of urine (the solution was turbid). Curve 2 acid alcoholic solutions each cubic centimeter of which contains an amount of hematoporphyrin representing 0.00125 cc of blood. Curve 3 hematoporphyrin equivalent to 0.25 cc of blood added to 1000 cc of urine. Curves 4 and 5 presence of hematoporphyrin in the urine of two persons tested.

All five curves of Fig. 5 were obtained spectrophotometrically from the acetic acid precipitates of 1000 cc specimens of urine. The precipitate in each case was dissolved in 25 cc of alcohol containing a small amount of hydrochloric acid. These solutions varied considerably in color or color attributes, as can be judged from the relative percentage transmission of light of various wave-lengths in the curves presented. In none of the curves, however, is there the slightest evidence of the presence of hematoporphyrin.

In an earlier portion of this paper, we stated that, in the case of an absorption zone definitely characteristic of the substance in solution, the concentration was proportional to the negative logarithm of the unabsorbed light. A solution of hematoporphyrin of known strength (equivalent to the hematoporphyrin from a given number of cubic centimeters of blood in a given quantity

of alcohol acetic acid solution), of which the spectrophotometric determinations are shown in Curve 2 of Fig. 4, was diluted in such a manner as to give definite fractional concentrations of the original solution. Tubes which were 5 cm. in length were used in obtaining the data of Fig. 6. The relationship between concentration and the logarithm of the unabsorbed light at wave length 555 millimicrons is a straight line as shown in Fig. 6 and therefore the substance in solution optically fulfills the laws of Lambert and Beer as expressed in equation 1. Since the value of the concentration of hematoporphyrin in the original solution is known it is possible to calculate from the curve of Fig. 6 the equivalent amount of hematoporphyrin in any solution that shows its presence provided the total volume of the solution is known.

CALCULATION OF THE AMOUNT OF HEMATOPORPHYRIN FOR EACH 1000 C.C. OF URINE

In Curve 2 of Fig. 4 each cubic centimeter contains the hematoporphyrin from 0.00125 c.c. of blood hence equivalent to the hematoporphyrin from 1.25 c.c. of blood for 1000 c.c. of solution. The value of the spectrophotometric reading at 555 millimicrons in Curve 3 of Fig. 4 is 60. By reference to Fig. 6 it may be noted that the value of the logarithm of 60 corresponds to a concen-

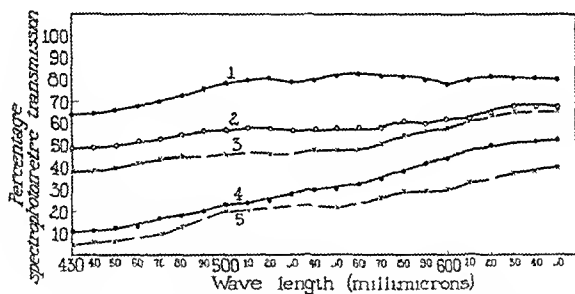


Fig. 5—Spectrophotometric curves of solutions made from acetic acid precipitates of 1000 c.c. of urine. There are no absorption bands characteristic of hematoporphyrin.

tration of $\frac{1}{100}$ of the original solution or the hematoporphyrin from 0.00040 c.c. of blood in each cubic centimeter of solution. This corresponds very well to the data given for Curve 3 of Fig. 4 which represents the recovery of hematoporphyrin from 1000 c.c. of urine equivalent to the hematoporphyrin from 0.25 c.c. of blood. Therefore 1 c.c. of urine contains the hematoporphyrin from 0.00025 c.c. of blood. In Curve 4 of Fig. 4 the reading at 555 millimicrons is 63. This is comparable to the corresponding reading of 60 in Curve 3. The volume of urine in the case represented by Curve 4 however was only 400 c.c. The ratio of the volume of urine for Curves 3 and 4 was $\frac{1000}{400}$ or 2.5:1. Therefore 1 c.c. of urine in the case represented by Curve 4 contains two and a half times as much hematoporphyrin as 1 c.c. of urine in the case represented by Curve 3. Hence, each 1000 c.c. of urine contains

hematoporphyrin equivalent to 0.625 cc of blood. In another set of determinations made on the hematoporphyrin in the urine of the same person the acetic acid precipitate from 500 cc of urine was redissolved in 10 cc of acidified alcohol. This solution, diluted one-sixth with alcohol and examined spectrophotometrically, gave a reading of 62 at 555 millimicrons. Knowing that the precipitate from 400 cc of urine redissolved in 25 cc of alcohol (Curve 4 of Fig. 4) gave an equivalent of hematoporphyrin from 0.625 cc of blood in each 1000 cc of urine, it can be concluded that the amount of hematoporphyrin was $\frac{400}{500} \times \frac{25}{10} = \frac{2}{1}$ as great as in the case of Curve 3, or that each 1000 cc of urine contained the hematoporphyrin equivalent to 1.25 cc of blood.

If such tests were to be indulged in as a matter of routine in clinical practice it is likely that a given aliquot portion of a twenty-four-hour specimen of urine (for example, 500 cc) would be taken in each instance, and

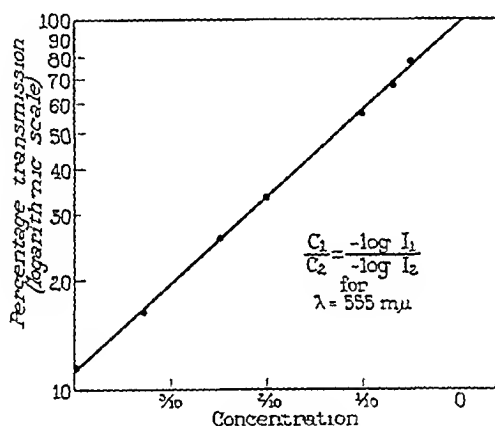


Fig. 6—Relationship between the logarithm of the percentage transmission of light for wave length 555 millimicrons and concentration of an alcoholic solution of hematoporphyrin

recovery of hematoporphyrin made by precipitation with acetic acid with subsequent solution of the precipitate in a given quantity of alcohol and acid (for example, 25 cc). Having as a standard, therefore, a given quantity of hematoporphyrin prepared from a known volume of blood and added to a given quantity of urine, followed by precipitation with acetic acid and re-solution in acidified alcohol (for example, the hematoporphyrin prepared from 0.25 cc of blood and added to 500 cc of urine with the subsequent acetic acid precipitate redissolved in 25 cc of alcohol) it is possible to obtain a curve such as that shown in Fig. 6, showing the relationship between concentrations and transmissions of light at 555 millimicrons. Therefore, keeping original volumes of urine and alcoholic solutions of precipitates constant quantities, it is possible to read off directly from the semilogarithmic chart, showing the relationship of concentration to percentages of transmitted light, the equivalent amount of hematoporphyrin in terms of cubic centimeters of blood for each cubic centimeter of urine or for each 1000 cc of urine.

SUMMARY

In this paper, in addition to some points regarding the theory and practice of spectrophotometry, we have presented (1) an outline of a method for the preparation of solutions of hematoporphyrin when present in urine, which affords a characteristic absorption zone spectrophotometrically, (2) spectrophotometric curves of normal urine and of urine containing hematoporphyrin, (3) methods for the calculation of the amount of hematoporphyrin in 1000 c c of urine in terms of the equivalent number of cubic centimeters of blood for each 1000 c c of urine, (4) applications of the laws of Lambert and Beer to the spectrophotometric data obtained with dilution and at wave length 555 millimicrons, the point of maximal absorption of alcoholic solutions of iodized acetic acid precipitates of hematoporphyrin in urine and (5) suggestions regarding a simplified clinical procedure

METHOD FOR STAINING OF POLAR BODIES*

By EMIL WEISS, M D, CHICAGO, ILL

A NUMBER of methods^{1 2 3} have been devised for the demonstration of polar bodies. The Neisser method is not always satisfactory. In this method the brief application of solution A prevents a deeper staining of polar bodies because the solution of methylene blue is very weak, the polar bodies are easily overstained by the contrasting dye. The acetic acid in the same solution only slightly swells the polar bodies and at the same time decreases the staining power of the already weak methylene blue. Therefore the contrasting staining of polar bodies and bacteria is not very definite. Neisser later recommended a modification which only partially removes the above defects. In this modification the contrasting stain for bacteria is better selected, but the polar bodies remain the same. These deficiencies in Neisser's procedure induced a number of workers to develop new methods of their own. Some of them brought about only slight modifications, which hardly offer any advantages over the original method with a resultant limited use. None of these modifications fulfill all the requirements (1) distinct staining of polar bodies, (2) enlargement of polar bodies, (3) distinct contrasting staining of bacteria, (4) simplicity of procedure.

The author thought it worth while to attempt a modification of the Neisser method by taking advantage of the improvements of some of the newer methods and adding to them certain steps which were found to be very efficient.

THE NEW METHOD

I Staining Solution for Polar Bodies—Solution A in the Neisser method contains 0.1 gram of methylene blue, 5 c.c. of glacial acetic acid, 2 c.c. of absolute alcohol and distilled water q.s. 100 c.c. In our modification the following changes are made: a high grade methylene blue (Gruber) was selected in a fifty times higher concentration (5 per cent) than in the original method (0.1 per cent). The swelling effect of the glacial acetic acid is considerably increased by a longer application of the above solution (five minutes). The content of alcohol is slightly increased (10 per cent). No difference was found in using absolute or 95 per cent alcohol. The proposed staining solution for polar bodies has the advantage that the polar bodies are more intensely stained and appear much larger than in the original method. The usefulness of methylene blue was compared with some other dyes (malachite green, thionine blue, brilliant green and gentian violet). No advantages were found in replacing methylene blue by any of the above dyes.

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II Staining Solution for Bacterial Bodies—Bismarck brown which was used in the original method was discarded because this dye does not keep well and the bacteria do not stand out plainly. The contrasting stain in Neisser's newer method easily overstains the polar bodies. Much attention has been paid to the selection of the best contrasting stain for methylene blue. Safranin and fuchsin were found to be most suitable. These dyes are on hand in every laboratory and the maintenance of a special second stain for this procedure is not necessary. A 1 per cent solution of safranin or fuchsin is ordinarily used. By diluting these dyes 1:20 the resultant contrasting staining is found to be most effective. A ready made solution of these dyes can easily be prepared by dissolving 0.1 gram of safranin or fuchsin in a solution made up of 180 c.c. of water and 20 c.c. of 95 per cent alcohol. This solution should be filtered and is then ready for use. This stain is applied for one to two minutes after washing off the previous solution.

DISCUSSION

The proposed method for the staining of polar bodies represents a modification of the Neisser method and has the following advantages over the latter: it increases the size of the polar bodies and stains them deeper; it shows up the bacterial bodies more plainly. The stains keep well and the technique is simple. The proposed method also favorably compares with other methods for staining of polar bodies.

SUMMARY

A new method has been devised for the staining of metachromatic bodies with the following procedure: the smears are fixed on the flame as usual. The slides are covered for five minutes with solution I (Gruebler's methylene blue 5 grams, 95 per cent alcohol 10 c.c., glacial acetic acid 5 c.c., distilled water q.s. 100 c.c.). The stain is then removed with running water and the slides are covered for one to two minutes with 1 per cent safranin or fuchsin 1:20. The slides are washed with water and dried. The metachromatic bodies appear deep blue; the bacterial bodies are distinctly red.

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A NEW CENTRIFUGE TUBE FOR VOLUME INDEX DETERMINATIONS (MODIFIED HADEN METHOD)*

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WE HAVE found the Haden method the most satisfactory for volume index determinations. There are, however, some objections to the use of the ordinary 15 c.c. centrifuge tube as suggested by Haden†. A tube made of ordinary glass often breaks at the high speed necessary for complete packing of erythrocytes. The other objection to this type of tube is the conical shape of the lower end, all the cells are often packed in this portion, and it is difficult to make an accurate reading. Then too all the graduations are too close together on a 15 c.c. tube to enable accurate reading.

To obviate these difficulties we have devised the tube illustrated‡. It is made of pyrex glass with heavy walls. It is 5 inches long and will fit in the 15 c.c. centrifuge shell. The capacity of the tube is only 6 c.c. so that the graduations in one-tenth cubic centimeters are well separated and the level of the packed cells is easily seen. The walls are parallel throughout the entire graduated portion thus making the reading of volumes at all levels equally easy.



Fig. 1

It is obvious that in using this tube just half the original Haden quantities are used in performing the volume index test. However, the following directions for carrying out the entire procedure may be found useful.

TECHNICAL PROCEDURE

The anticoagulant used by Haden is 1.6 per cent sodium oxalate. Exactly 1 c.c. measured with a pipette is placed in the volume index tube. Blood is withdrawn by venipuncture in a dry syringe and placed at once in the tube to exactly the 6 c.c. mark. In other words, exactly 5 c.c. of patient's blood should be used for the test. The tube is inverted to mix the blood with the oxalate solution. An erythrocyte count must also be made as near the time of the venipuncture as is possible.

The tube is then centrifugalized for forty-five minutes at about 2500 revolutions each minute or until maximal packing of cells has taken place. No-

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†Haden R. L. Clinical Laboratory Methods, St. Louis, p. 104, 1923. C. V. Mosby Co.

‡Manufactured by Arthur H. Thomas Co., West Washington Square, Philadelphia, Pa.

mal blood, containing 5,000,000 cells, will divide into 46 per cent to 48 per cent cellular elements, and 54 per cent to 52 per cent plasma. We have found in our centrifuges that the normal figure is generally 48 per cent and the cells will pack to the 24 cc mark, so we use this figure for 100 per cent. The volume index is

$$\frac{\text{volume per cent}}{\text{erythrocyte per cent}} \quad \text{or} \quad \frac{\text{cell volume} - 24}{\text{erythrocytes} - 5,000,000}$$

In pernicious anemia, with cells larger than normal, the index is more than 10 and in secondary anemia, with smaller cells predominating, the index is less than 10.

We have found Table I useful in determining the volume per cent.

TABLE I

VOLUME PER CENT OF ERYTHROCYTES IN 5 CC BLOOD TAKING 48 IFB CFAT OR 24 CC AS NORMAL

VOLUME OF ERYTHROCYTES, CC	PER CENT
240	100.0
235	98.0
230	96.0
225	94.0
220	91.5
215	89.0
210	87.5
205	85.0
200	83.3
195	81.0
190	79.0
185	77.0
180	75.0
175	73.0
170	70.8
165	68.6
160	66.6
155	64.5
150	62.5
145	60.5
140	58.0
135	56.0
130	54.0
125	52.0
120	50.0
115	48.0
110	45.8
105	43.7
100	41.5
95	39.5
90	37.5
85	35.7
80	33.0
75	31.0
70	29.0
65	27.0
60	25.0
55	23.0
50	20.8
45	18.4
40	16.3
35	14.3
30	13.2
25	10.4

A MODIFICATION OF THE SIMPSON METHOD OF FROG HEART PERFUSION*

BY ELKIN VOGT, B S, AUGUSTA, GEORGIA

THE following modification of the Simpson method of heart perfusion was devised in order to make use of hearts of small frogs weighing approximately twenty grams and even less. For reasons which will be apparent to the reader, it was also used for hearts of large frogs as well.

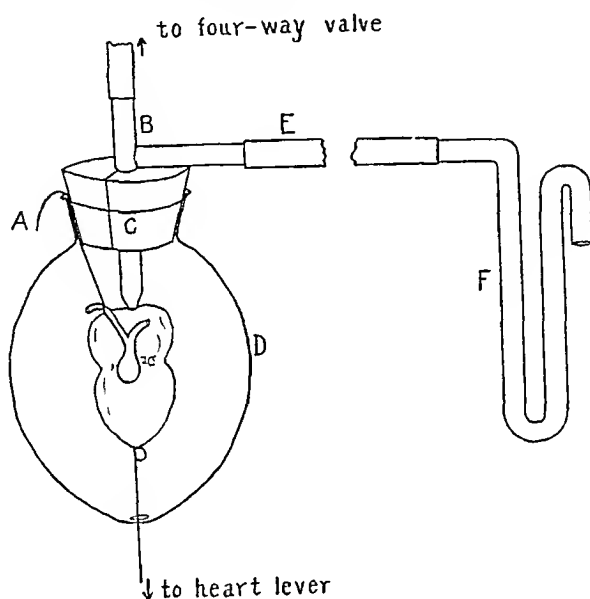


Fig 1 —Diagram of mounted heart and pressure regulating U-tube Reduced $\frac{1}{2}$

After the heart is exposed, a ligature (A, Fig 1) is tightly tied near the base of the right branch of the aorta. One end of the ligature is not cut off, as it is to be used in suspending the heart. A waxed thread is then tied to the apex of the heart, the ventricle lifted, and a small glass cannula (B) with a side arm is inserted into the vena cava near the sinus venosus and tied securely. The left branch of the aorta is severed, close to the bulbus, or a small hole is cut in the bulbus, and the heart quickly washed out with Ringer's solution, before removal, by allowing the solution from a pressure bottle to enter the inlet cannula. This prevents the formation of blood clots within the heart. The heart is removed and a rubber stopper (C), bearing a hole in the center for the cannula and a slit from the hole to the periphery, is placed

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around the cannula immediately below the side arm. The stopper is then inserted into a glass tube or the funnel part of a small thistle tube (D) in such a manner as to catch between the stopper and the tube the end of the ligature around the right branch of the aorta. The ligature is now adjusted so as to aid in suspending the heart and prevent its being pulled from the inlet cannula. The heart is mounted with the cannula joining a four way valve by a short rubber tube. The side arm of the cannula is then connected by means of a rubber tube five or six inches long to a small water manometer or U shaped tube (F) which has its free end at the top bent sharply downward. The inflow pressure is now regulated to permit sufficient distention and perfusion of the heart. It was found that good results were obtained when the heart was placed from three to four centimeters below the

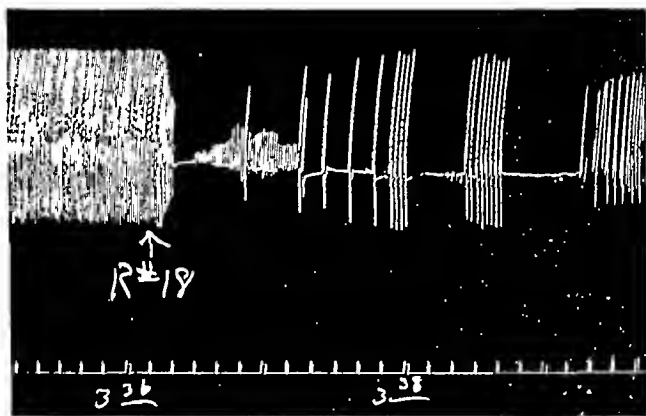


Fig. —Record of contractions of the heart of a twenty gram frog. Note there is no tendency to distention during the quiescent periods. The heart was receiving approximately twelve drops of perfusate per minute while inactive.

level of the fluid in the reservoir. The U tube is then raised or lowered until the perfusate drops slowly from its open end. The manometer by permitting the perfusate to escape from its open end prevents the distention of a heart which has ceased to beat. That stretching the muscle fibers of the heart modifies the action of drugs has been shown by Hunt (1915) and more recently by Barlow (1928). Changes in the tension of the heart muscle also occur when the fluid in the reservoir is renewed from the supply flask and when the heart varies in amplitude and force of contraction. The effects of such changes are recorded more readily by small than by large hearts, and the manometer described considerably reduces these effects. It is necessary that the relative levels of this U tube and of the heart be such that not only is distention of the quiescent heart prevented but also that a pressure sufficient to force the fluid through the ventricle is maintained. The proper posi-

tions can be determined only by a few trials with the particular apparatus being used

An aortic cannula is not used and, therefore, the drops from the aorta are not generally recorded. However, if desirable, the rate of perfusion may be registered from the drops leaving the end of the string tied to the heart lever. Since a heart properly mounted according to this method receives sufficient solution at all times, the recording of the rate of perfusion seemed an unnecessary technical procedure and was therefore discontinued. It was found by experiments in this laboratory that a small heart, mounted as described, beats more vigorously and for a longer time, and responds more promptly to a change of perfusate, than a heart mounted with an aortic cannula inserted.

The lever used is that of the Harvard type. To get the best results this must be correctly balanced, especially when using a small heart. The rest of the apparatus is essentially that described by Simpson (1911). A four-way valve, devised by Quigley and Heath (1925), is also used.

The advantages of this method are

- 1 The hearts of small frogs may be used
- 2 The heart is rapidly and easily mounted
- 3 Manipulation of the heart is reduced, as no aortic cannula is used
- 4 Drying of the heart is prevented and no moist chamber is necessary, as the perfusate passes over the outside as well as through the heart. It is best, however, to use an enclosing tube to prevent air currents from reaching the heart and to afford an easy means of suspending the heart by the ligature mentioned

5 The perfusate reaches the heart quickly and elicits prompt response

6 A quiescent heart receives ample perfusate, as no aortic cannula is present to retard the flow

7 The side U-tube prevents the extreme distention of a heart stopped in diastole and also diminishes other variations in tension of the heart muscle

SUMMARY

1 A modified method of perfusion, adapted to small frog hearts, is described

2 A means of preventing the extreme distention of a quiescent heart is also given

3 Advantages of the method are enumerated

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A MODIFICATION OF THE MORSE KOPELOFF ANAEROBIC METHOD*

By P. A. TETRAUIT, PH. D. MADISON, WIS.

IN 1922 Morse and Kopeloff¹ devised a simple plate method for anaerobic culture. Halves of Petri dishes, exactly matched as to diameter, are sterilized. Inoculated agar is poured into one half of the chamber and allowed to harden. The chamber is then inverted and a pyrogallie acid all ali mixture placed in the lower half. The two halves are then sealed together with adhesive tape and the chamber is ready for incubation. For long periods of incubation, it is recommended that the tape be varnished to make it more impervious to air.

The same principle is apparent in the method to be described but it involves certain improvements which make it more generally applicable for bacteriologic work.

In the culture of thermophilic organisms it was found that adhesive tape could not be used. It does not give an airtight seal nor does the tape adhere long at the high temperature necessary for incubation. Furthermore, it is rather expensive for routine work. Heavy gummed paper tape gives a much better seal, and there is no trouble with peeling off. The cost is a negligible item. The use of pyrogallie acid and all ali as described by Morse and Kopeloff has one very serious disadvantage. When plates are stacked in the incubator, a slight jar will cause the mixture to splash over the surface of the agar and ruin the culture. This is overcome in our method by placing a piece of sterile absorbent cotton in the bottom of the chamber.

McLeod, 1913 designed a plate involving a special porcelain chamber for the absorbent, and a modified Petri plate for the culture. The two are sealed together by means of plasticine. This plate was tried but was discarded as the plasticine soon dries at high temperatures making a perfect seal impossible. Further, the plate is so expensive that it cannot be used for ordinary laboratory work.

The method as modified and used is as follows:

The inoculated agar, gelatin, or silica gel is poured into one half of the Petri dish. This is covered with another half Petri dish of the same diameter, and the medium is allowed to solidify. The chamber is then inverted. In the lower half is placed a piece of sterile absorbent cotton. On this cotton is poured 10 cc of 20 per cent KOH and then 3 cc of 44 per cent pyrogallol. The chamber is immediately closed and sealed with paper tape. The tape should be of the heavy variety coated on one side with fish glue. The thin variety has been tried but does not give satisfactory results. The tape is wound three or four times around the chamber.

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According to Riemsdijk³ 10 c.c. of 20 per cent KOH and 3 c.c. of 44 per cent pyrogallol are sufficient to absorb the oxygen from 400 c.c. of an incubation in thirty to forty minutes. The volume of a chamber measuring 9 cm. in diameter and 3 cm. in depth is a little less than 200 c.c. The proportion suggested by Riemsdijk will more than take care of this volume. At 28° C. and 37° C., this excess may be used provided the incubation period does not exceed one week. This concentration of KOH will, with long periods of incubation, dehydrate the medium. As the temperature of incubation is raised, dehydration is more rapid so that either the incubation period has to be shortened or the concentration of KOH reduced. At 60° C., 10 c.c. of 10 per cent KOH and 1.5 c.c. of 44 per cent pyrogallol have worked out very satisfactorily. Plates have been kept at this temperature for two months with little or no dehydration of the agar.

This plate is also adapted for anaerobic culture using slices of vegetable tissue in place of pyrogallol and alkali. Where CO₂ is essential to bacterial growth, slices of potato, turnip, etc., are recommended. At 28° C. and at 37° C. this procedure gives very good results but at higher temperatures, the vegetable tissue is not satisfactory. Perhaps the temperature is too high to allow respiration of the tissue.

The plates are opened by first removing the bulk of the paper with a knife and then inserting a razor blade between the dishes and cutting through the remainder of the paper.

Experiments were carried out at 28° C. using butyric acid organisms, at 37° C. using butyric acid and butyl alcohol organisms as well as *B. sporogenes*, and at 55° C. to 60° C. using thermophiles from various animal manures. All gave equally good results, well isolated colonies being obtained within thirty-six hours.

Other than the advantages already listed by Morse and Kopeloff, the following are offered:

1. Use of gummed-paper tape. It offers a more impervious seal. It does not dry out at high temperatures.
2. The use of slices of vegetables. The anaerobic chamber is well adapted for this type of experimentation.
3. Use of standardized base and pyrogallol. Varying degrees of anaerobiosis may be obtained by varying the amount of each.

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A USEFUL MEDIUM FOR CULTIVATION OF THE GONOCOCCUS AND ALLIED ORGANISMS*

By ROBB SPALDING SPRAY, PH D,† MOROANTOWN W VA

BACTERIOLOGIC literature is already so burdened with formulas for special media for the isolation and cultivation of microorganisms that it would seem superfluous to propose additions. However, when one is devised which may be quickly and easily prepared upon which the gonococcus may be grown without the necessity of blood or serum it would seem appropriate to call it to the attention of those who in the small clinical laboratory, may not have the time nor facilities for obtaining sterile blood.

The formula and methods given below are based upon the principle of the "hormone" medium of Hinton and of the well known North's agar, and have been modified from a procedure outlined by D B Carter¹. All of the necessary ingredients are easily obtained and no special equipment is required in its preparation.

The underlying principle which may be commonly known is that fresh meat infusion contains a property variously termed "hormone," "vitamine," or better "growth necessary factor" which is adsorbed if the infusion is passed through organic matter such as cotton gauze, wool or charcoal. In the making of this medium glass wool only is used when filtration is required which is but once.

The formula and details of preparation are as follows:

To 500 gm fresh, lean fat free veal add 1 liter of preferably distilled water (chlorine free tap water is satisfactory). Boil five minutes with continuous stirring to prevent scorching or steam thirty minutes in a double boiler. Pack the stem of a glass funnel loosely with glass wool and lay in about one inch more. Pour off the infusion and meat into funnel, refiltering the first 100 cc, or until clear. Allow all infusion to drain off and make up to exactly one liter. This constitutes the stock infusion.

Instead of fresh veal a dehydrated veal powder (Bacto Veal) may be substituted with perfect satisfaction. In this case 50 gm of powdered veal are infused in 1 liter of water at about 50° C for one hour then boiled five minutes, or steamed for thirty minutes in double boiler as above and the infusion filtered through glass wool. Much time and effort are saved by the use of this dehydrated meat.

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Director of Laboratories Swan Myers Biological Company Indianapolis Indiana.

*Prepared by Digestive Ferments Company Detroit Michigan

To 1 liter of this stock infusion add

Peptone (Difco Bacto)	20 gm
Sodium chloride (C P)	5 gm
Nutrose (Sodium caseinate, Difco)	2 gm
Gelatin (Crystalline)	20 gm
Soluble Starch (or Corn Starch)	10 gm

Boil five minutes, with continuous stirring to avoid scorching. The medium is strongly acid and must be adjusted with NaOH. Phenolphthalein may be used for titration, and the reaction adjusted to plus 0.1 or 0.2 acidity, or better, using the newer method, titrate with phenol red to P_H 7.4 or 7.5.

After titrating and adjusting reaction add

Agar (Shred)	15 gm
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Boil until agar is dissolved. With a good grade of agar the reaction is not appreciably affected. Measure in cylinder and make up to 1 liter with water.

Place in a small cylindrical container so that a fair depth is obtained, cover loosely with a lid, and autoclave for fifteen minutes at 15 pounds pressure. Allow to cool slowly in the autoclave until solid, when the cylindrical mass may be turned out on a table. The lower layer will contain a heavy sediment which may be cut away and discarded leaving perfectly clear agar. This is melted and distributed in plugged tubes or in flasks and again autoclaved. The plugged flasks may then be paraffined and kept until required for slants or plates.

There is a slight loss in discarding the bottom layer of precipitate, but this is more than compensated for by the saving of time required in filtration or clearing with egg.

To this medium may be added carbohydrates, glycerin or blood, if desired for special purposes, and all of the common pathogens, as well as the more delicate ones such as gonococcus, meningococcus, pneumococcus and influenza bacillus (with addition of heated blood) grow abundantly on initial isolation.

For the preparation of autogenous vaccines, or for mass cultivation for antigens, this medium is vastly superior to plain agar. One slant of this medium will give the equivalent growth of three plain agar slants of *Staphylococcus aureus* within twenty-four hours.

For the small laboratory this complete medium is now obtainable in dehydrated form² ready for preparation involving only the weighing of 7.7 gm of powder, adding 100 c.c. of distilled water, boiling about five minutes, tubing and autoclaving. This prepared medium appears to be in all ways equal to that which is freshly prepared, and the simplicity of its preparation renders it a very useful, and readily available medium for all general, and most special requirements.

AN EXPLORATION LLECTRODE TO DETERMINE THE HYDROGEN ION CONCFNIRATION OF FLUIDS IN LIVING TISSUE*

By HENRY LIHRENBURG, D Sc., SAN FRANCISCO CALIF

WHILE determining the hydrogen ion concentration of plant fluids, an electrode has been developed which makes possible these determinations directly on the plant. The instrument works equally well for the determination of the P_H of healthy and diseased tissue in man and animal. It can be brought directly in contact with a focus of infection or with the secretion of a gland.

The instrument to be described can readily be made in the laboratory. The sketches (actual size) show the simplicity of the parts and are to a great extent self explanatory. The letter *A* designates the electrode point and *B* the electrode holder which has a hydrogen inlet and a rubber connection *E*. The latter keeps the electrode *C* in place and prevents the escape of hydrogen. *D* shows the lower half of the electrode assembly connected with an agar agar KCl bridge. It is in position and in contact with the fluid to be measured. The contact is made possible by the glass tube of *C* being larger than the capillary of *B*. On stopping the flow of hydrogen and raising *C* in *B*, liquid is pulled up in the capillary through a greater height than the electrode moves. Raising the electrode thus until contact is established the correct E.M.F. can at once be measured.

It is needless to emphasize that the usual precautions, temperature pure hydrogen correct set up, and functioning of the remainder of the apparatus must be observed. Tight rubber connections are essential. A pointer galvanometer (No 2320 Leeds & Northrup) in connection with a saturated KCl calomel electrode is sufficiently sensitive to produce an easily noticeable deflection for a 0.01 P_H , and with a magnifying glass for a 0.005 P_H . Considering the influence of temperature variations alone the accuracy gained by closer reading is illusory.

For explorations an $\frac{1}{8}$ inch rubber tube of suitable length with a glass point filled with saturated KCl agar agar serves as a flexible bridge connecting the calomel electrode. A thin copper wire connects the hydrogen electrode with the potentiometer. The electrode is held by a long movable clamp attached to a support and can thus be moved from place to place.

ELECTRODES

To work well and to get good results the electrodes must be one millimeter thick, not less, and made either of platinum or pure gold. Thinner wires give a variable and sinking E.M.F. A 7 mm length of the wire is

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welded to a $\frac{1}{4}$ mm thick platinum wire, a simple operation with a pair of smooth, flat pliers. A tiny piece of gold foil is a good solder for platinum. The wire can also be drawn out. It is essential that the unplatinized part of the wire in the glass tube be as thin as specified, and be short, in order not to abstract too much hydrogen from the platinized part which would also result in a low E M F. An electrode thus made will keep a constant E M F for hours. The thick end is cut off smooth and square. If the thin end is fused into the glass tube *C* of the size illustrated, then the mercury will not run out when the tube is inverted. A coat (thin) of palladium is preferable to platinum because it is more easily removed. This is important for gold or gold-soldered electrodes, since the chlorine liberated by electrolysis dissolves gold readily.

The contractions of the electrode holder must be well centered and must fit the electrode snugly so that its point cannot chafe against the glass wall.

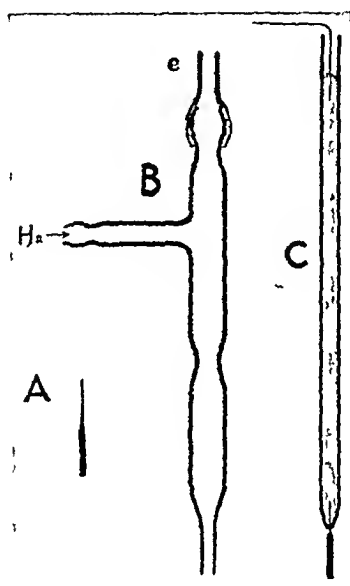


Fig 1

of the capillary and rub off the black deposit. With proper care a great number of determinations can be made before repalladizing, itself a very simple and short operation.

OPERATION

A gentle stream of moist hydrogen is passed through the electrode holder immersed in a check buffer of known P_H . After all air has been washed out the hydrogen is cut off and the electrode raised until the entering buffer touches the point. The correct E M F signifies that it functions properly. The capillary, with bridge and point is now flushed in distilled water by pinching the hydrogen rubber connection. The electrode is then moved to the tissue surface. By depressing the tissue slightly, as in Fig 2, fluid gathers there. This is drawn into the capillary and its E M F measured. Repeated contacts should be made to be sure that it is constant. If the moisture is insuffi-

cient a drop of distilled CO free H₂O is added. A better way still is to immerse the tip of the electrode capillary into conductivity water, bubble a little hydrogen through it, seal the capillary with a drop of water and then put it in position. The electrode is manipulated or the rubber connection pinched so that the drop works in and out care being taken to exclude bubbles of air. The drop will mix with the sap and its P_H can be determined. Where the available moisture is minute, as on a ligament or the surface of an organ, the dilution with even one drop of water is enormous. If this drop contains impurities inaccurate E.M.F.'s will result. A low P_H is caused by CO. The correct value may be attained if the CO is blown out by a gentle stream of hydrogen. This is possible in many instances. With pure water no

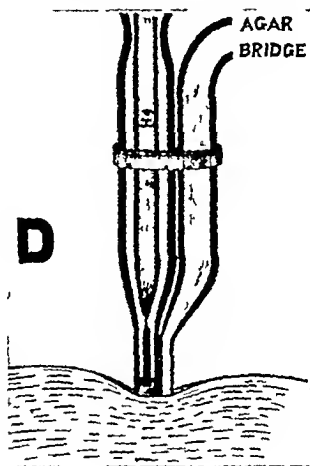


Fig. 2

difficulties will be encountered and the correct E.M.F. is easily obtained. At the first trial with the electrode on the peritoneum of a mouse, the P_H remained for over an hour at 7.46.

The fluids of living animal tissue are very rich in buffers and also are highly ionized. Both these properties are of great advantage. The richness in buffers allows of considerable dilution; it enables the attainment of an immediate, constant E.M.F. usually at the first contact; the high ionization makes possible a large current and a correspondingly big deflection of the galvanometer for a minute difference in E.M.F.

With a properly functioning electrode, the correct E.M.F. is established immediately on contact; hence the NaHCO₃/CO equilibrium in blood is not likely to be disturbed. Thus its P_H can possibly be determined directly at the point of incision. Since the CO escapes into the atmosphere, however, no

equilibrium of the E M F is attainable and one may be doubtful which value is correct. The trials made as yet are too few to warrant the formation of any opinion.

The E M F of the hydrogen electrode is determined by the concentration and temperature of the hydrogen and the concentration of the hydrogen ions. In the point electrode the temperature is that of contact with the fluid measured. The P_H is calculated from the well-known formula

$$E M F = E - \frac{RT}{nF} \log \frac{C_1}{C}$$

where E M F is the electromotive force indicated by the potentiometer,

E the potential of the calomel electrode

and $\frac{RT}{nF} \log \frac{C_1}{C_2}$ the expression for the hydrogen potential

Disregarding the + or - signs, nonessential in this problem, we get

$$E M F = E + 0.0591 \log C \quad (H_2^{25^\circ})$$

$\log C$ is the P_H

As examples for different temperatures for the hydrogen electrode and saturated KCl calomel electrode let

$$E M F = 0.683v$$

Then with the calomel and hydrogen electrodes at the temperatures indicated

$$\begin{aligned} (1) \quad E M F &= E^{25^\circ} + 0.0591 \log C \quad (H_2^{25^\circ}) \\ 0.683 &= 0.246 + 0.0591 (\chi) \\ \frac{0.437}{0.0591} &= \chi = 7.4 = P_H \end{aligned}$$

$$\begin{aligned} (2) \quad E M F &= E^{37^\circ} + 0.0616 \log C \quad (H_2^{37.5^\circ})^* \\ 0.683 &= 0.246 + 0.0616 (\chi) \\ \frac{0.437}{0.0616} &= \chi = 7.1 = P_H \end{aligned}$$

$$\begin{aligned} (3) \quad E M F &= E^{37^\circ} + 0.0616 \log C \quad (H_2^{37^\circ}) \\ 0.683 &= 0.235 + 0.0616 (\chi) \\ \frac{0.448}{0.0616} &= \chi = 7.3 = P_H \end{aligned}$$

Working at such a high temperature as 37° the temperature coefficient of the standard cell, Weston or Clark, and the increased resistance of the potentiometer, calibrated for 25° , must not be neglected. Considering the errors all these corrections imply, it is far better to work in a room at 25° , get the temperature factor for the hydrogen electrode by measuring the E M F of a strong buffer of the P_H range involved at 25° and at 37.5° and apply the difference.

*Mislowitz. Die Bestimmung der Wasserstoffionenkonzentration p 167-170 1928
Clark The Determination of Hydrogen Ions p 314 1928

A CONVENIENT MODIFICATION OF THE BIURET TEST*

By DAVID H. KLING, M.D., LOS ANGELES, CALIF.

THE Biuret test is still regarded as the most important color reaction for proteins and protein derivatives; therefore, the publication of a convenient modification seems justified.

Technic—One c.c. of Haines qualitative solution is placed in each of two Wassermann test tubes. The solution to be tested for protein is then added, drop by drop, to one of the Wassermann tubes. From 5 to 20 drops are sufficient. A purplish, violet or blue ring will develop at the point of contact of reagent and solution in the presence of albumin or derivatives. At the same time, a reddish hue appears in the overlying solution. In case the ring formation is not definite, mix the contents by shaking the test tube. Natural albumin gives a deep blue or purplish color. Peptones and polypeptides give a pinkish slightly red tinge.

The second test tube is useful as a control to detect the positive reaction if it consists only of a change to a deeper blue.

A special Biuret reagent can be made up by combining 9 c.c. of 10 per cent sodium hydrate and 1 c.c. of 1 per cent copper sulphate. The test is then conducted in the same manner as outlined above. The color is lighter and more of a pinkish red. However, this reagent is not stable because a precipitate of cupric hydrate forms after a few days.

This modification offers the following advantages:

1. The standard test for Biuret requires two reagents: the first a strong alkali, the second, a weak (1 per cent) copper sulphate solution. The latter has to be made up for this reaction and added with great care, drop by drop to the unknown solution in order that the blue color does not cover the reaction.

The outlined modification requires only one reagent which is widely used for sugar detection and, therefore, is on hand in most laboratories.

2. This modification is conducted as a microreaction and requires therefore very small amount of the unknown solution.

3. The modification is conducted as a "ring test."

4. The result is checked by the use of a control.

Of the other commonly used sugar reagents, Benedict's qualitative reagent can be adapted to this reaction by diluting with a double amount of 10 per cent sodium hydrate. Fehling's reagent can only be used by combining 2 c.c. of Solution B, or an equal amount of 10 per cent sodium hydrate with two drops of Solution A (copper sulphate). Neither of these reagents is so satisfactory as Haines solution.

417 TOWNE AVENUE.

From the Laboratory of the Golden State Hospital
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A MODIFICATION OF THE ORSKOV SINGLE-CELL TECHNIC*

By D C B DUFF, M A, TORONTO, CANADA

ORSKOV¹ has described a simple and efficient method of obtaining single-cell cultures which has been used with success by Crowell² and others. It is the purpose of this communication to describe certain modifications of the original method, which seem to do away with the chief objection that has been raised to this method, i.e., that, during the actual picking off of the microcolony which has developed from a single cell, the operator must lose sight of the field, owing to the necessity of substituting a dummy objective on which the needle is mounted.

The essentials of the original¹ procedure are as follows. A dilute saline or broth suspension of the organism is spread upon an agar plate. A block of agar is removed, with precautions as to sterility of technique, from a portion of the plate where it is thought the individual bacilli will be reasonably separated. The block is mounted upon a sterile glass slide, the surface of which has previously been scored with fine lines by means of a diamond pencil. The slide is placed in a graduated mechanical stage of a microscope. By using a high power "dry" lens, a single cell is located and centered in the field of vision. The location on the mechanical stage is noted. The high power lens is then replaced by one of lower magnification, which is focussed down upon the complex of fine lines on the surface of the glass slide, and immediately below the agar block. A careful sketch is made of the manner in which the lines intersect, this sketch is later used to relocate accurately the site. The slide is then removed from the microscope and placed, with adequate provisions for moisture, in the incubator for a few hours until microcolonies have formed. The slide is then replaced on the stage. The original site is first roughly found by the calibration of the mechanical stage, and then accurately by relocating the complex of lines indicated in the sketch. On focussing up from these lines a microcolony should be found exactly in the center of the field. This colony has developed from the original single cell. The objective is then replaced by another upon which a sterile needle has previously been mounted, with wax or plasticine. The tube of the microscope is then lowered until the needle touches the colony, and is again raised. The organisms adhering to the needle may be wiped off by a loop of sterile broth and inoculated into a suitable medium. A subsequent examination of the field is made to see that the needle has disturbed only the colony desired.

This method, while entirely practicable, has disadvantages which have been overcome by certain changes in the procedure, the most important of which, as will be seen below, is the system of picking off the microcolony under direct observation, by means of an apparatus of the Chambers micromanipulator type³. The detailed procedures are given below.

*From the School of Hygiene University of Toronto
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1 A supply of finely and irregularly scored glass slides marked in one corner to ensure proper orientation are kept in 95 per cent alcohol and are removed and flamed as required. A supply of sterile capillary pipettes or needles for use in the micromanipulator unit must also be on hand.

2 From 10 to 12 cc of a sterile 2 per cent nutrient (infusion) agar, containing 1 per cent dextrose is poured into a Petri plate. In working with *C. diphtheriae* the agar may be enriched by the addition of sterile normal serum.

3 A fairly thin suspension (about No 1 or No 2 McFarland scale) the proper density of which can be determined only by experience is made in nutrient broth. The suspension should be made from an eighteen to twenty-four hour culture into broth at incubator temperature and should not be allowed to cool. In the case of organisms such as *C. diphtheriae* which tend to hold themselves in clumps the suspension is passed through a coarse alundum filter the procedure being carried out at incubator temperature. The resulting suspension contains practically nothing but single cells.

4 One or two loops of the filtered suspension are placed at the periphery of the agar plate and spread in the usual manner with a sterile glass spreader. The spreader passes only once over the plate so that the organisms are comparatively close together at one side and are far apart at the other. The plate is then placed in the incubator for about one hour. It has been found¹ that a preliminary incubation increases the refractive quality of the bacteria and renders them easier to see.

5 A strip of agar slightly less in length and width than the scored slide is cut with a sterile knife from the plate so that a gradation in the number of organisms per unit area will be found from one end to the other. This is mounted on the flamed slide the scored side of which is upward taking care that no air bubbles are left between agar and slide.

6 The mounted preparation is placed on the mechanical stage and searched with a high power dry lens for single cells which are well separated from their adjacent neighbors. An area on the slide can quickly be found containing a large number of single cells suitable for isolation. In this laboratory a Zeiss binocular microscope is used with Zeiss K 15X oculars a Reichert 6b objective for searching and a lower power objective for drawing lines.

As many as ten isolations may be made from one strip of agar. It is not advisable to make more as during this time the organisms are exposed to the drying effect of air currents and are not at the proper temperature. It is preferable to work in a fairly warm room without drafts.

7 After the cells have been accurately located as explained above the slide is placed in a Petri plate containing moist blotting paper and incubated for a period of twelve hours until microcolonies barely visible to the naked eye are formed. These colonies may be seen without much difficulty at the thickly seeded end of the strip.

8 The slide is replaced properly oriented upon the mechanical stage and one of the single cell colonies is relocated as rapidly as possible. The low power No 3 objective is then used to give sufficient clearance between lens

and again for the introduction of the tip of a pipette mounted in a micro manipulator unit. The colony is picked off by the pipette under the direct observation of the worker, and the tip of the pipette or needle is broken into a small amount of serum broth, which should be at incubator temperature. The quantity of broth should not exceed 2 c.c. By the use of amounts greater than this, the number of successful growths from isolations is greatly decreased.

9. The procedures outlined in No. 8 are repeated as rapidly as may be for each colony known to have grown from a single cell. It is then possible to make ten isolations from one preparation of which from five to eight will produce successful growth.

While the employment of the Orskov method, as modified above, required care and accuracy in its execution, it seems to demand no abnormal degree of technical skill, and a very short period of practice suffices to produce a successful operator. As compared with even the most carefully worked out systems of the "hanging drop" type, it appears considerably simpler and very much more rapid.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDFEE, M.D., ABSTRACT EDITOR

BILE The Important Microscopic Elements In Bile Hollander E. *Am J Med Sc* 177 371 1929

In the microscopic examination of bile obtained from the gall bladder and from the bile ducts four elements are found that are diagnostic of pathologic states of the biliary tract: bile flocculi, intensely bile stained debris, agminated cholesterol crystals and sandlike particles.

These elements were found in bile both from the gall bladder and from the ducts with the exception of agminated cholesterol crystals which occurred only in the gall bladder.

GRAM REACTION The Cell Wall and the Gram Reaction Burke V. and Barnes M. W. *J Bact* 18 69 1929

The Gram reaction of yeast cells is due to and influenced by the same factors that determine and influence the Gram reaction of bacterial cells.

The bacterial cell has a definite cell wall. After the cell is crushed and the protoplasm extruded the empty sac can be seen. In appearance it resembles the empty sac of a crushed yeast cell. Both are unstained after exposure to the Gram stain.

The breaking of the cell wall causes the protoplasm of gram positive bacteria and of yeast cells to become gram negative. The protoplasm within intact cells and the extruded protoplasm may have the same I_{H} and stain differently. The extruded protoplasm cannot be made gram positive.

Acid and alkali readily penetrate the cell wall. They interfere with the Gram reaction by affecting the passage of the dye iodine precipitate through the cell wall. They alter the affinity between dye and protoplasm but this is not part of the Gram reaction though it may interfere with it to a minor extent. Acid and alkali, as well as water, probably affect the Gram reaction by altering the permeability of the cell wall.

The function of the mordant in the Gram technique is that of a precipitating agent. The precipitate must be insoluble in water, soluble in the decolorizer. It must less readily pass through the cell wall of the gram positive organisms. The precipitation of the dye results in its losing its affinity for the protoplasm.

The affinity of the aqueous gentian violet for both gram negative and gram positive bacteria or the extruded protoplasm can be increased by hydrogen peroxide and alkali. Protoplasm so treated resists decolorization. This reaction has been confused with the true Gram reaction. It is nullified by the action of Gram's iodine. The authors have designated this reaction as the pseudo gram reaction. Since hydrogen peroxide and alkali are quite different agents it is possible that more than one reaction is involved in what we have termed the pseudo gram reaction.

Any factor that alters the cell wall or the dye iodine precipitate may affect the Gram reaction. The protoplasm of the cell plays no part in the Gram reaction. Bacteria are gram positive, gram negative or gram variable depending upon the permeability of the cell wall.

MENINGOCOCCUS Isolation and Cultivation of Gosling R. *J A M A* 93 611 1929

To nutrient real infusion broth 1 per cent of dextrose and 0.25 per cent of agar are added. The reaction is adjusted to pH 7.2 to 7.4. The mixture is tubed in 10 c.c. amounts and sterilized in the Arnold apparatus one hour on two successive days. It should be firm enough when cold to hold its shape when gently inverted, should offer no resistance to the needle and should be semisolid when at incubative temperature.

The meningococcus appears on the surface within from eighteen to ninety six hours

The inoculum is thoroughly blended with the upper half inch of the medium and a deep stab made for anaerobes

POLYMORPHONUCLEAR COUNT IN NEWBORN Sanford, H N Am J Dis Child 38 271, Aug, 1929

Sanford reports upon the Cooke and Ponder modification of the Arneeth count in infants From a study of 60 infants he concludes that

1 The polymorphonuclear leucocytes of the newborn show a preponderance of the single and double lobed nucleated forms

2 Only a few of the trilobed polymorphonuclear cells exist at birth, and none of the other multilobed forms appear until the ninth day

3 There is a rapid tendency toward the adult mean

A study of the weighted mean (cells in Class I multiplied by 1, Class II by 2, Class III by 3, and Class IV by 4 Add and divide by total number of cells, normal adult average 2.74) shows that the polymorphonuclear count of the newborn undergoes rapid readjustment as the cells mature On the first day, when there is such a preponderance of young, single lobed cells of Class I, the mean is only 1.43 This increases rapidly until the tenth day, when it is 2.07, indicating a marked tendency to arrive at the stable adult mean of 2.74

VOGES PROSKAUER REACTION Bedford, R H J Bact 18 93, 1929

The culture medium consisted of proteose peptone, glucose, and dipotassium hydrogen phosphate, which was inoculated with a known positive test organism, and incubated at 37° for a definite period of time To 25 cc of the culture medium, 10 mg of sodium peroxide were added and immediately afterward 1 cc of 40 per cent sodium hydroxide, the culture tube was then placed in boiling water for one minute, and then shaken vigorously In less than one minute the color became perceptible

ADAPTATION OF THE METHOD TO LABORATORY PRACTICE

Instead of weighing out 10 mg of sodium peroxide, a sufficient amount can be held on the end of the wire of an inoculating needle, the loop of which has been bent to the shape of the letter "M," or any other solid surface will do just as well This is then placed in the culture medium and gently warmed over the Bunsen flame, at the same time constantly agitating the contents of the tube

The addition of too great a quantity of the sodium peroxide will cause further oxidation of the direct compound and consequent loss of color Any doubt as to the positivity of the test can be set at rest by pouring the contents of the culture tube into a white porcelain, evaporating dish, the white background makes a more definite contrast and any color can be readily detected This has been found a convenient adjunct in the laboratories

BLOOD SUGAR Effect of Liver on, Blattner, H, and Murphy, W P J A M A 92 1332, 1929

A study of the effect of liver feeding on the blood sugar indicates that whereas previously liver has been regarded as an unsuitable article of food for diabetic patients because of its glycogen content, it is now known to have a beneficial effect on the blood sugar of these patients

The liver fractions that are effective in the treatment of pernicious anemia have no effect on the blood sugar, whereas certain liver fractions that are ineffective in the treatment of pernicious anemia have no effect on the blood sugar like that of liver

Four patients with diabetes taking liver daily or from three to five times a week have been observed with repeated blood sugar determinations for approximately one year, while in two who were followed for twenty and thirty days it was found that the blood sugar had remained at a constantly lower level than previous to liver therapy

These observations suggest that liver contains a blood sugar reducing substance active when taken by mouth, nontoxic and with an effect on the blood sugar concentration similar to that obtained with insulin.

It is difficult to estimate the quantity of liver that will replace a known amount of insulin, but the authors feel that 180 gm of liver will have an effect on the blood sugar of certain diabetic patients equal to that of from 10 to 15 units of insulin.

RETICULOCYTES Response of to Liver Therapy Minot G R Murphy W P and Stetson R P *Ann Int Med* 4: 175-551 1929

The anemia of pernicious anemia is primarily dependent upon the failure of the primitive cells in the bone marrow to differentiate toward mature erythrocytes.

In pernicious anemia there occurs with extraordinary regularity a prompt, temporary, pronounced increase of the reticulocytes in the peripheral blood following the administration of large amounts of liver, kidney or potent fractions of liver. The number of reticulocytes usually remains definitely elevated for about nine days.

The reticulocyte response which has been studied in more than 100 cases of pernicious anemia is ascribed to the specific active principle effective in pernicious anemia promoting the growth of the primitive cells that crowd the bone marrow in relapse probably hastening or permitting their maturation.

The height to which the percentage of reticulocytes rises in the usual case is in inverse relation to the level of the red blood cells. Cases with more than 3 million red blood cells per cmm exhibit never more than a slight response of the reticulocytes. In essentially all cases however treated with adequate amounts of active principle, the red blood cells rapidly increase to normal.

Accompanying the response of the reticulocytes other immature types of formed bone marrow elements may appear in the blood and the white blood cells and platelets increase in numbers. Very large amounts of effective material may favor the appearance of many immature bone marrow cells.

Small amounts of effective material or complications of the disease may cause only a slight rise of the reticulocytes which may be delayed.

When submaximal quantities of liver have been fed the effect of additional amounts will be reflected in the course taken by the reticulocytes. A weak response may not soon be followed by significant increase of the total number of red blood cells. If treatment is commenced when there is a considerable spontaneous increase of reticulocytes or when this has just occurred no subsequent rise of these cells may follow. The feeding of large amounts of liver produces no response of the reticulocytes in normal persons. Liver therapy in many cases of "secondary" anemia is a different matter than the treatment of pernicious anemia.

A reticulocyte response to liver therapy may occur in other cases of anemia than pernicious anemia but it is absent or slight in ordinary cases of "secondary" anemia. Some unusual cases and some cases of anemia associated with pregnancy have shown a marked response of the reticulocytes like that seen in pernicious anemia. This has also occurred in sprue and in anemia due to the fish tapeworm.

TUBERCLE BACILLI Primary Culture of A Simple Glycerol Water Crystal Violet Potato Cylinder Medium for Diagnostic Cultures Corper H J and Uyel N *Arch Path* 7: 835, 1929

The following simplification of the original technique is described.

One cubic centimeter of suspected material is beaten to a homogeneous pulp and introduced into a sterile centrifuge tube of 15 cc capacity with 1 cc of 6 per cent sulphuric acid (containing 1 cc of 96 per cent [specific gravity 1.84] sulphuric acid in 500 cc of distilled water) and mixed. After incubation at 37 C for thirty minutes the contents of the tube are mixed with about 10 cc of sterile 0.9 per cent sodium chloride solution and

centrifugated. The residue, after the supernatant fluid has been decanted, is seeded on the surface of the glycerol water crystal violet potato cylinder medium, the culture tube being capped with tin foil after the cotton plug has been lightly impregnated with hot paraffin to prevent drying out of the medium. The medium is prepared by placing 15 cc of 6 per cent aqueous solution of glycerol (made with pure tap water or distilled water) in a sterile culture tube, 6 inches by $\frac{3}{4}$ inch (15.24 by 1.9 cm) in size, in which has been inserted the crystal violet potato cylinder, about 3 inches (7.6 cm) long and $\frac{5}{8}$ inch (1.59 cm) in diameter. The latter made by soaking a clean potato cylinder halved longitudinally, in a freshly mixed 0.0015 per cent standard crystal violet in 1 per cent sodium carbonate solution (prepared from the pure anhydrous salt). The entire medium is sterilized in an autoclave at 15 pounds (6.8 Kg) pressure for thirty minutes. Excessive or prolonged heating of the medium during sterilization is to be avoided. The culture tubes should be incubated in the dark with due precaution being taken to avoid drying of the medium or contamination. A luxuriant growth should occur on this medium within from two to six weeks, but if the culture is negative, the tubes should not be discarded for diagnostic purposes until after three months' observation at incubator temperature.

MEDIA Sodium Chloride Media for the Separation of Certain Gram Positive Cocci from Gram-Negative Bacilli, Hill, J H., and White, E C. J Bact 17 43, 1929

It has been found that P_H 6.0 sodium chloride agars, with salt concentrations from 2 through 20 per cent, exert marked inhibitory action on the growth of bacilli of the typhoid, paratyphoid, dysentery, and colon groups, on species of *Proteus*, *Pseudomonas*, on diphtheroids, and on *Bacillus anthracis*. The gram positive cocci studied tolerate high salt concentrations, all being positive on transfer from 20 per cent sodium chloride agar.

In P_H 6.0 broths, with salt concentrations from 2 through 25 per cent, the same differential bacteriostasis may be observed, although to a lesser degree than on agar.

It has been found that when mixtures of cocci and bacilli in different proportions are cultured on appropriate salt agars, the cocci invariably outgrow the bacilli and may some times be recovered in pure culture.

The use of 6, 8, 10 and 15 per cent salt agars greatly facilitates the isolation of gram positive cocci from specimens from mixed infections.

The use of such salt agars is therefore suggested for the inhibition of gram negative bacilli and for the isolation of gram positive cocci.

TISSUE Staining of Tumors of Spongioblastic Origin, Foot N C. Am J Path 5 239, 1929

Material Frozen sections of tissue fixed in 10 per cent formalin are cut 15 to 20 microns, thickish sections are more easily manipulated without tearing and the processes of the spongioblasts, which may branch in all directions, are less apt to be cut off in the plane of section.

Bromuration The sections are washed in distilled water and bromurated by the Globus technique, being placed in 10 per cent ammonium hydroxide for twenty four hours, washed briefly in distilled water and transferred to a 10 per cent solution of 40 per cent (concentrated) hydrobromic acid, where they remain for five to twenty four hours. They are then transferred to Cjajal's formalin bromide (3 gm NII₂Br in 15 per cent neutral formalin, 100 cc) where they may be stored until wanted. Although the ammonium bath is supposed to serve as a deformalizing agent, it is found that the sections become more surely impregnated if they have been in formalin bromide for a few hours. The tissue sections should be handled, throughout the process, with a glass spatula, made by bending about one centimeter of the end of a glass rod to an angle of 60 to 80° and pinching it out thin and flat while still hot and soft, with warmed pliers, so that its broad surface runs transversely, like a scoop. This avoids the tearing of sections so common when glass needles are used, and it facilitates their transfer from bath to bath.

Impregnation The sections are washed in two changes of distilled water and placed in about 35 c.c. of weak silver carbonate solution which has been heated to about 45° C. The vessel is then set in an incubator at 38° C. for half an hour. The silver solution is made up by mixing 50 c.c. of 10 per cent AgNO_3 with an like amount of 5 per cent Na_2CO_3 , shaking, allowing the heavy white precipitate to settle and decanting the supernatant fluid. The precipitate is then washed in 200 c.c. of distilled water and again allowed to settle. It is next nearly dissolved, after decanting most of the supernatant fluid by adding strong ammonium hydroxide drop by drop and continually shaking the graduate until only a few grayish granules remain undissolved. The solution is then made up to 200 c.c. with distilled water and stored in a brown bottle where it will keep for days. If a precipitate forms in the bottle it should be filtered off. The used silver diamino carbonate solution however must never be poured back into the bottle. The sections become reddish brown in this bath.

Reduction After washing them in two changes of distilled water they are brought into a 1 per cent neutral formalin for 2 to 3 minutes. In a few seconds they become ochraceous gray to black and two minutes is usually enough time for complete reduction.

Toning They are then washed in water tap water is no longer inadmissible after reduction is complete and toned for two to three minutes in a 1:500 solution of gold chloride. This keeps well and may be used repeatedly until it no longer removes the yellows and browns from the color scheme of the sections which indicates that it is exhausted. I use Merck's brown acid AuCl_3 the cheaper yellow salt will probably serve just as well.

Fixation After washing the sections are fixed in 5 per cent sodium thiosulphate for two to five minutes which removes the metallic luster from their surface takes out superfluous metallic salts and renders the tissue pliant.

Mounting After washing away the hypo each section is mounted upon a clean glass slide by being floated over it one corner pinned to the slide with the glass spatula and the slide removed from the water at a distinctly obtuse angle bringing it out at an angle too acute with the surface of the water creates eddies that wrinkle the section. Loose or crumpled edges are brought out flat on the glass by immersing the slide edgewise deeply enough in the water to float them free holding it at right angles to the surface and moving it gently up and down until all ribs and loose ends have been straightened out. It is then blotted firmly with several layers of smooth unpatterned filter paper and blotting usually results in the section adhering to the paper rather than to the slide. If the sections be blotted firmly enough it will be found that no egg albumen glycerin adhesive need be used on the slides. The section is then anchored by the Mallory and Wright collodion method. It is flooded with 95 per cent alcohol from a dropping bottle then with absolute alcohol and once more blotted dry. A few drops of very thin collodion are then flowed over the section and gently blown upon until they set in a thin film. The process is then reversed a few drops of absolute alcohol followed by 95 per cent alcohol are dropped over the collodion and the slide placed in a staining dish of water. The collodion should be just thick enough to flow in drops and to set in the thinnest possible film that will serve to anchor the sections firmly to the glass. A little experience will teach one how much collodion should be dissolved in ether with a little absolute alcohol added to insure complete solution.

Counterstaining The sections may now be handled in slotted glass staining dishes like paraffin sections. They are stained for five minutes in a fresh solution of Harris hematoxylin and washed in water until they become blue. Working the water or adding a few drops of ammonia will hasten the bluing. They are then stained for thirty to forty five seconds in Van Gieson's stain (10 c.c. of 1 per cent aqueous acid fuchsin to 90 c.c. saturated aqueous picric acid solution). The stain is poured off as completely as possible and the sections run directly up through 95 per cent and absolute alcohol (omitting a wash in water) into xylol with 5 per cent oil of origanum. After a brief immersion in pure xylol they are mounted.

RESULTS OF THE METHOD

Nuclei Brownish red to red mitotic figures brighter red

Cytoplasm Excepting that of the astronevres yellowish red to brown

Astrocytes The fibrillary type will show black fibrils and a faint red cytoplasm the

protoplasmic type will be almost entirely black, save for the nucleus "Irritation" astrocytes are gray to black Vascular processes (sucker feet) are deeply impregnated and black

Spongioblasts The cytoplasm is reddish yellow or brownish, the polar filaments black The apolar variety may show "fibrogenic areas" in black if they be mature enough Giant cells stain chiefly with the hematoxylin Van Gieson stain, showing little affinity for silver

Nerve Cells These are distinctly brownish, with a few black dendrites and blackish Nissl bodies

Oligodendroglia and Microglia Although sometimes well impregnated, these cells are not specifically stained, and the method is not yet recommended for their demonstration **Gitterzellen** Black network on yellowish brown background

Neuroglia Fibers Black

Fibrous Reticulum Black

Collagen Fibers Bright vermilion

Erythrocytes Canary yellow to brown

Granulocytes and Monocytes These usually show very distinct argyrophil granules

Areas of hemorrhage and necrosis are very clearly demonstrated and one may thus gauge the presence or absence of degenerative changes very accurately It is to be noted that the bloated, snow shoe shaped spongioblasts and the swollen and vacuolated Gitterzellen occur chiefly in the neighborhood of necrotic areas and represent degeneration forms Pseudorosettes are well brought out

WATER BACTERIOLOGY The Eijkman Fermentation Test as an Aid in the Detection of Fecal Organisms in Water, Leiter, L W Am J Hyg 9 705, 1929

As originally carried out by Eijkman, the water to be examined was mixed with $\frac{1}{8}$ of its volume of an aqueous solution of 10 per cent glucose, 10 per cent peptone and 5 per cent sodium chloride, placed in a fermentation tube and incubated at 46° C *Bacillus coli*, if present, overgrew the other bacteria and usually within twenty four hours was found in pure culture or in almost pure culture The solution was highly turbid in both the open and closed arms and the sugar fermented with gas formation

Pure waters, free from any suggestion of fecal contamination, did not ferment the Eijkman solution at 46° C, even in such large quantities as 300 cc and usually only a slight turbidity of the medium was evident For the examination of contaminated waters fermentation tubes of 5 to 10 cc capacity were filled with a nutrient solution containing 1 per cent glucose and small quantities of water added At 46° C a positive reaction was obtained in some instances with such small quantities as 1/100 cc

The following formula is used

Dextrose	10 per cent
Peptone	10 per cent
Sodium chloride	5 per cent
Water	75 per cent

The medium was sterilized in the autoclave at 10 pounds pressure for ten minutes at 110° C In carrying out the Eijkman test 1 part of the medium was diluted with 7 parts of water In the examination of small quantities of water (10 cc) ordinary fish hook fermentation tubes were used With larger quantities, a 250 cc flask was employed, equipped as follows

A two hole stopper was fitted with two U tubes the one reaching to the bottom of the flask, the other just through the stopper The outer ends of both tubes were then drawn to a thread and sealed With stopper in place, the flask was sterilized in the autoclave at 15 pounds pressure for fifteen minutes

When the flask was used, the sealed tip of the shorter tube was broken, the flask filled with the proper proportions of medium and suspected water The stopper was then

replaced with pressure sufficient to expel all the air from the flask. The tube was resealed in a flame and the sealed end of the long tube broken. The flask was then placed in the incubator with the open tube over a beaker to receive the fluid expelled in the process of growth by the formation of gas in the top of the flask. Gas formation can thus be fully observed in the examination of quantities of water up to 15 c.c. For the examination of smaller quantities the quantity of water to be tested was put in the flask to which was added the amount of medium required for the total volume of the flask. The flask was then filled with sterile water. Any quantity of water can thus be tested by the employment of flasks of suitable size equipped in a similar manner.

In testing the ability of pure cultures of organisms to grow in the Eijkman dextrose peptone broth the medium was diluted to the concentration employed in the analysis of water, namely 1 of medium to 10 parts of water. The following formula yields a corresponding concentration:

Dextrose	12.5 gm
Peptone	12.5 gm
Sodium chloride	6.25 gm
Water	1000 gm

The medium was tubed in fish hook tubes and sterilized in this case at 20 pounds for twenty minutes.

The following conclusions are presented:

The Eijkman Fermentation Test at 46° C. applied to water is selective for *Bacillus coli* and inhibits or destroys other organisms ordinarily present in water.

Strains of *Bacillus coli* obtained in pure culture from warm blooded animal feces grow and produce typical gas acid and growth reactions in Eijkman's dextrose peptone broth at 46° C. as a fairly uniform and constant characteristic.

Certain organisms answering the description of *Bacillus coli* and isolated from the intestines of cold blooded animals fail to produce a typical reaction in Eijkman's dextrose peptone broth at 46° C.

A close correlation exists between the fermentation of Eijkman's dextrose peptone broth at 46° C. the production of indol, the nonutilization of sodium citrate and the non utilization of the nitrogen in uric acid by strains of *Bacillus coli* known to have been isolated from warm blooded animal feces.

The Eijkman Fermentation Test at 46° C. is usually complete in sixteen to twenty four hours and is thus more rapid than standard methods.

In a high percentage (92.75 per cent) of the positive Eijkman Fermentation Tests of water the presence of *Bacillus coli* can be confirmed in comparison to the low percentage (78.40 per cent) of the positive lactose tests of standard methods.

Positive Eijkman tests of water yield members of the aerogenes cloacae group infrequently in marked contrast to the frequency of their isolation by standard methods.

Waters freely inhabited by cold blooded animals although removed from the possibility of contamination by warm blooded animal feces may be condemned by standard methods and be passed by the Eijkman test.

TISSUE Silver Staining of the Skin and Its Tumors Laidlaw G. F. Am. J. Path. 5 239, 1929

Formula Bouin's Fluid (Masson's Formula) To 300 c.c. tap water add 100 c.c. commercial formal and 20 c.c. glacial acetic acid. Add an excess of picric acid. Shake frequently and keep an excess of picric acid in the fluid. Ready for use in three days and keeps indefinitely.

Masson's Gelatin Glue Dissolve 0.05 gm. gelatin (in practice, a bit of ordinary sheet gelatin 5 millimeters square) in 20 c.c. distilled water warming it over the flame. Place a row of slides on the warm plate. Filter a large drop of gelatin solution on each slide and float the paraffin section on it. As soon as the section spreads, stand the slide upright to

dram, holding the section for a moment in the desired place with a brush or needle. At this stage do not permit the section to dry, when the excess gelatin has drained off, blot the section and place immediately in the oven at 45 to 50° C in formol vapor, secured by leaving in the oven in open dish of formal. For staining with hematoxylin and aniline dyes, twenty minutes in the hot formal vapor is sufficient, for silver staining, leave the slides for several hours or, better, overnight.

Ten Per Cent Lithium Silver (Modified del Rio Hortega) To make 120 cc. In a 250 cc glass stoppered graduate, dissolve 12 gm silver nitrate sp in 20 cc distilled water.

Add 230 cc saturated solution lithium carbonate sp in distilled water, shake well, let settle to about 70 cc of precipitate, wash well with distilled water 3 or 4 times.

After settling to about 70 cc of precipitate, decant the wash water, add aqua ammonia fortior, shaking constantly until the fluid is almost clear.

Add distilled water to a total of 120 cc, shake and filter into stock bottle. The solution keeps for many months. It is so strong that a slight precipitate is negligible.

Ordinary filter paper is apt to turn brown and discolor the solution while filtering. Use Whatman filter paper No 42 or No 44 or Schleicher and Schull No 589.

1 Fix in Bouin for three days or in 10 per cent neutral formal for three days. Old formal material may be improved by immersion in fresh neutral formal for three days. Formal fixed tissue immersed in Bouin for three days will give nearly perfectly but not quite perfect Bouin pictures, a positive endothelial or smooth muscle nucleus here and there betrays the original form fixation.

2 Embed in paraffin or celloidin, or make frozen sections.

3 Stick paraffin sections on the slide with Masson's gelatin glue and harden in hot formal fumes, sections so treated seldom float off.

4 After removal of the paraffin, wash Bouin sections in running water for twenty minutes to remove the picric acid, wash formal sections for five minutes.

5 Mordant with the Mallory bleach. Tissue recently fixed in formal (two to ten days) often gives the reaction without mordanting but not constantly.

(a) 1 per cent tincture of iodine, three minutes, rinse in tap water.

(b) 5 per cent hypo (sodium thiosulphate), three minutes, rinse in tap water.

(c) ¼ per cent potassium permanganate, three minutes, rinse in tap water.

(d) 5 per cent oxalic acid, three minutes, wash well in running water for ten minutes.

6 Distilled water, change 3 times within five to ten minutes to ensure clean slides entering the silver solution.

7 Rio Hortega's lithium silver augmented to 10 per cent. Heat the stock solution in the oven to 50° C and stain in the oven for five minutes. The used solution can be filtered into a glass stoppered bottle and used a dozen times or more.

8 Rinse the slides by pouring distilled water over both sides.

9 Formal, 1 per cent in tap water, flood the sections frequently for three minutes.

10 Rinse both sides of the slide with distilled water.

11 Yellow gold chloride, 1 to 500 of distilled water in a Coplin jar, immerse the slides at room temperature for ten minutes. The gold solution may be used many times.

12 Rinse both sides of the slides with distilled water.

13 Oxalic acid 5 per cent, pour on the slide and leave for ten minutes.

14 Rinse with distilled water.

15 Hypo, 5 per cent, pour on slide. Change as often as it becomes turbid for ten minutes.

16 Wash well in running water. Counterstain if desired and mount in balsam.

The Mallory bleach solution and the formal may be left on the slides for an hour or more without harm. Even the silver solution may vary in strength from 2 per cent to 15 per cent, the time in silver from two to 10 minutes and the temperature of the silver solution from room temperature (twenty minutes) to 60° C, though the longer times and the higher temperatures are likely to give precipitates and dirty slides. After the formal, the slides may be left overnight in gold or in oxalic acid, and at any point in the technique the slides may be left overnight or over Sunday in distilled water or in tap water.

TUBERCLE BACILLUS Medium for Corper, H J and Uyel N Arch Intn 7 855, 1929

The following medium is proposed for subculture after isolation by the potato gentian violet method of Corper

Mashed autoclaved potato	25	per cent by weight
Glycerol -----	25	per cent by weight
Agar agar - - -	15	per cent by weight
Distilled water -	71	per cent by weight

TISSUE STAIN A Method of Staining Brown and Melanotic Pigment, Lasnier E P Virch Arch f path Annt 266 693 1928

The sections should be thin (3 to 5 micra)

Stain

Ziehl's Carbol Fuchsin 1 part diluted with 20 to 30 parts of tap water

The sections are stained $\frac{1}{4}$ to 1 $\frac{1}{4}$ minutes depending on whether they are frozen celloidin or paraffin sections They are then washed with water until the excess of stain is removed this requires some seconds but the stain is not injured by longer irrigation Strong staining with reduced hematoxylin (hematin) follows

The sections are washed for a few minutes in water that is changed a couple of times It does not hurt them to stay longer in water but on the contrary improves them as it gives the hematoxylin a beautiful blue shade The differentiation and drying are brought about at the same time This is the delicate moment of the technique It is advisable not to make the differentiation under the microscope for decolorizing takes place very quickly and the whole thing may become decolorized while the specimen is being carried to the microscope It is advisable to sacrifice a half dozen sections in the beginning until the examiner learns to differentiate from the naked eye appearance The water is removed by drying as well as possible with filter paper The sections are then flooded with 95 to 97 per cent alcohol The alcohol removes the excess of fuchsin and as soon as there is no longer any visible red color removed (between four and six seconds) and the violet blue color of the hematoxylin appears the sections are flooded with xylol The excess of xylol is sponged off and the specimens cleared with xylol and enclosed in balsam

The nuclei appear violet or deep blue the protoplasm blue or pale violet, the pigment bright red The fibrils of the heart muscle appear reddish violet to lilac violet depending on the degree of their differentiation the sarcoplasm is lilac or very pale violet almost unstained The pigment granules are bright red the erythrocytes often have a rosy shade but in well differentiated specimens they have a greenish lilac color

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EDITORIALS

The Polomyelitis Epidemic in Manitoba—1928

IN DISEASES which show as much variation in severity as polomyelitis, it is very difficult to evaluate the effect of any particular form of treatment. Controlling the results and calculating the probable error are almost impossible. One of the nearest approaches made to the solution of this difficult situation is presented in the cooperative investigation published by the Medical Research Committee of the University of Manitoba which deals with the recent epidemic of polomyelitis in Manitoba. It is evidently the first of a series of publications to be presented by the Department of Health and Public Welfare.

The epidemic began July 1, 1928, and lasted four and a half months, during which time there were 435 cases within the province, of these, 302 were in Winnipeg or its suburbs. There were thirty-seven deaths (8.5 per cent). The report contains a complete review of 161 cases which were especially observed

Of these patients seventeen died fifty four had residual paralysis and ninety recovered completely.

The most significant part of the study consisted in observations on the effect of the administration of convalescent serum. This was first observed in human beings by Netter following the observations of Romei and Joseph that convalescent serum contains immune bodies and following the further demonstration by Flexner and Lewis that such serum delays or may prevent the onset of the disease in experimentally inoculated monkeys. Since Netter's observations several investigators have reported favorably on the use of convalescent serum in the treatment of poliomyelitis.

Serum was obtained from volunteer donors and was paid for at the approximate rate of \$10.00 for each 100 cc. of blood. These donors had had the disease from a few months to thirty three years previously. After pooling the serum it was for the most part administered intramuscularly in doses of 25 cc. The administration of more than one dose seemed not to increase the beneficial results over the effect of a single dose.

Seventy four patients in whom the diagnosis was confirmed by spinal puncture received serum when they were in the preparalytic stage of the disease. Of these slightly more than 93 per cent completely recovered about 7 per cent had some residual paralysis and none died. Of thirty three patients who received serum after the onset of paralysis 22 per cent recovered completely, 45 per cent had residual paralysis and 33 per cent died. Of fifty four patients who did not receive serum 26 per cent recovered completely 63 per cent had residual paralysis and 11 per cent died. Although it is of course impossible to determine that the treated and untreated patients were absolutely comparable evidence is presented to show that they were in no way intentionally selected and the great difference in the end results seems significant. For instance, of the patients who had early paresis or paralysis 62 per cent of those treated recovered, only 20 per cent recovered in the control group. Of patients who had early paresis alone 67 per cent of those treated and only 40 per cent of the untreated recovered. Not only is the end result of importance but the immediate effect of the treatment is impressive. Within a few hours there was a drop in temperature and recovery from most of the symptoms of which the patients complained. The conclusion seems justified that the use of convalescent serum is of value when administered in the preparalytic stage and that the intramuscular route of administration is 'simple safe and sufficiently efficacious to justify its use during an epidemic.'

Further observations are of interest and may be summarized as follows.

No significance could be placed on the fact that the disease was more prevalent among males than females. The evidence presented indicated nothing for or against the transmission of the disease by food air animals or otherwise, except by contact with persons by some medium or mode yet to be demonstrated. It is safe to infer that when this contact occurs the onset of the disease appears from five to seven days afterward, hence the desirability of rigid isolation of persons suspected of having, or having the disease.

The greatest incidence of the disease was in children of from five to ten years but among children less than five years of age the incidence was almost

as great. In the country there was a relatively larger number of patients more than fifteen years of age than in the urban population. The distribution of cases in Winnipeg was central, while certain densely populated areas were practically free.

The greatest number of deaths occurred between the ages of five and nine years. Whether patients were treated or untreated the prognosis was, as a general rule, more serious when the cell counts were high. Seventy-nine per cent of the spinal fluids examined (116 cases) showed a cell count of between 10 and 200 cells for each cubic centimeter, the highest number was 1809 cells. In the early stages of the disease most of the cells were polymorphonuclear leucocytes.

Albuminuria and the presence of erythrocytes in the urine were frequently noted. In thirty of forty-three cases leucocytosis and lymphocytosis was the rule. A composite colloidal gold curve would read 0123210000.

Of the ninety patients who became paralyzed, fifty-one were paralyzed on the third day. More than 50 per cent of the patients had the following symptoms appearing in the following order: fever, frontal headache, stiff sore neck and back, and lumbar pain. Most of the symptoms appeared within the first three days. Absent abdominal reflexes were observed in one-third of the cases and adenitis in one-fifth. Kernig's sign was present in more than one-third of the cases, the spinal sign, in three-fourths. Knee jerks were absent in one-third and paresis or paralysis, in half of the cases.

—T B M

ERRATUM

In the article by Barclay E. Noble, entitled *Experimental Elimination of Adhesions Caused by Intraperitoneal Injection of Neosphenamine*, October issue, in the table on page 21, under the column head "Peritonitis" there should be the following qualifying footnotes. In the cat series the peritonitis consisted of 10 c.c. of a sterile fluid and evidence of recent adhesions, in the glucose series the peritonitis was localized to a small peri-intestinal abscess, in Ringer's solution series the peritonitis was purely chemical, and, in the sodium bicarbonate series the peritonitis was secondary to the intussusception. In the entire series, no generalized bacterial peritonitis was produced.

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Report of the Committee on Necrology

Four times during the past year has death invaded the ranks of this organization and taken from us the communities they served the profession they loved and the friends and families they held dear those whom we regret greatly to lose. Four men have been cut down in the midst of their productive years and at the height of their usefulness. In these days when we can ill afford to lose men whose lives have been given to the promotion of clinical pathology, their loss is keenly felt.

The East the Central States and the West are represented in this loss. Dr Joseph Rankin Losee and Dr John Hewat both resided and worked in New York City. Dr Marinus Larsen Holm in Lansing Michigan and Dr Carl O E Werner in San Francisco.

Nature is apparently wasteful in some of her methods and nowhere is this apparent waste so evident as in the snapping off of the lives of those who have diligently labored and become wise by their labors and of great usefulness in the world. But though they rest from their labors their works do follow them, the memory of their lives and works will be a haven of comfort to those who follow in their train.

The Committee presents herewith biographical data upon each of these and requests that this report be placed upon our records and expressions of our sincere sympathy be sent to the respective families

J H BLACK, M D, *Chairman*, DALLAS, TEXAS

JOSEPH RANKIN LOSEE, M D

Dr Joseph Rankin Losee was born November 12, 1883, at Collins Bay, Ontario, Canada, and died February 6, 1929, at St Petersburg, Florida

His preliminary education was obtained in the public schools of Ontario and Queen's University at Kingston, Ontario, where he received a B A degree in 1904 He was graduated with the degree of Doctor of Medicine from the Long Island College Medical School in 1907

He served an internship in St John's Hospital, Brooklyn, New York, 1907 to 1909, then in the Hospital for the Ruptured and Crippled, 1909 He was House-Surgeon in the New York Lying-In Hospital in 1910, and Pathologic Intern in New York Lying-In Hospital in 1911 In 1912 he was appointed Pathologist to the Lying-In Hospital, which appointment he held until the time of his death

He was Director of the Laboratory and Executive Officer in the New York Polyclinic Hospital and was Professor of Pathology in the New York Polyclinic Hospital and Medical School

He was a member of the American Medical Association, the American Society of Clinical Pathologists, New York County and State Medical Societies, New York Academy of Medicine, New York Pathological Society, and Alumni Societies of New York Lying-In Hospital

JOHN HEWAT, M D

Dr John Hewat was born in Scotland and graduated from the University of Edinburgh in 1909

He served in a division of the British Army during the World War as Chief Sanitary Inspector in India, Arabia, and Mesopotamia

Later he was Laboratory Director for the State Board of Health in Maine for about two years From there he went to the Central Maine General Hospital at Lewiston, where he was Pathologist for two years

Following this he became associated with the National Pathologic Laboratories as Pathologist in New York City, and was engaged in this work at the time of his death which occurred on February 13, 1929

CARL O L WERNER MD

Dr Carl O L Werner was born in Strassburg in 1876. He studied medicine at the University of Berlin and Jena in Germany. He graduated from the latter in 1901.

He went to Singapore the following year where he practiced until 1915. This gave him a wonderful opportunity to acquire a widespread experience in the theory and practice of tropical medicine.



He came to San Francisco in 1916 where he specialized in Clinical Pathology. For ten years he was Pathologist to the Franklin Hospital and during the past year filled a similar position at the St. Joseph's Hospital in the same city.

In 1928 Dr. Werner was appointed Instructor in Tropical Diseases at the Medical School of Stanford University. This position he held at the time of his death.

Dr. Werner died February 21, 1929.

MARINUS LARSEN HOLM, M D

Dr Marinus Larsen Holm was born in 1878. He received his medical education at the Medical School of Northwestern University, from which he graduated in 1907.

He was City Chemist to the Chicago Health Department in 1906 and 1907 and State Bacteriologist of Michigan in 1907 to 1914.

Dr Holm served during the World War and later was a member of the City Board of Health of Lansing, and for a time a member of the Board of Education.



He was Director of the Lansing Clinical Laboratory, a member of the Staff of Ingham County Tuberculosis Sanitarium, and Pathologist to the Lansing City Hospital.

He was a member of the American Medical Association, the American Society of Clinical Pathologists, Society of American Bacteriologists, the American Public Health Association and the American Chemical Society.

Dr Holm died at his home in Lansing, Michigan, December 24, 1928.



VICTOR C VAUGHAN, M D
1851 1929

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CLINICAL AND EXPERIMENTAL

TUBERCULOSIS IN GUINEA PIGS*

A SKIN TEST FOR PREEXISTING TUBERCULOSIS IN GUINEA PIGS USED FOR
LABORATORY DIAGNOSIS OF TUBERCULOSIS

BY HENRY A REISMAN, M D JAMAICA N Y, AND ADELAIDE B BAYLIS
NEW YORK CITY

THE guinea pig is universally accepted as the animal of choice for the inoculation diagnosis of tuberculosis. Much reliance is placed on the outcome of this procedure. It is always accepted as conclusive in cases of doubt where some evidence of tuberculosis is found in the guinea pig on necropsy with little realization as to whether or not there was a preexisting tuberculous infection.

Two years ago we concluded experiments the results of which at least to us were surprising. We then found, much to our chagrin that we had been preceded in this work by at least a decade. In view of the fact that there is little cognizance of their results and conclusions and that experiments with guinea pigs are still being carried on without ascertaining whether the guinea pig is tuberculosis free, we feel that publication is quite apropos.

We also have reference to some of the work on the filterable virus in tuberculosis.⁴ The filtrate was injected into the guinea pig without eliminating a preexisting tuberculous focus. If there was such a focus present (and our work has led us to believe that in a large percentage of cases there is), the filtrate would activate that focus thereby causing an erroneous conclusion namely that that filtrate had produced the disease. This has its analogy in the tuberculin tests in cattle. Furthermore at that time (two years ago) we made a survey of the pathologic departments of all the larger hospitals in New York City to ascertain whether an attempt was made to eliminate a

From the Department of Pediatrics and the Department of Laboratories New York Post Graduate Medical School and Hospital

Received for publication June 26 1929

preexisting tuberculous focus in guinea pigs before doing the inoculation diagnosis for tuberculosis. In not one instance where we had received a reply was the procedure to eliminate an existing focus followed. It is obvious therefore that not only experiments may come to an erroneous conclusion, but that many human beings may be called tuberculous and serious operations performed such as nephrectomies when only the guinea pig had the tuberculosis. Sewal¹ found spontaneous tuberculosis in a notable proportion of guinea pigs which had been confined in the laboratory, but which had never been intentionally inoculated with tuberculosis. Cooper and Petroff² have demonstrated the presence of tubercle bacilli in lymph nodes in 33 per cent of normal guinea pigs. We therefore feel justified in having the following observations published.

Twelve young guinea pigs, supposedly healthy, weighing about 250 gm were selected for the first experiment and were placed on the floor of the animal room in an enclosure separating them from the other laboratory animals by means of a mesh wire fence. They were allowed to remain there for a period of four days for observation.

On February 26, 1927, an area on the abdomen approximately 2.5 cm in diameter was bared by plucking the hair so that any skin reaction would be apparent, and the following day the 12 guinea pigs were tested with 0.25 cc of a 1-10 dilution of Koch's old tuberculin by intradermal inoculations. After twenty-four hours four of the 12 guinea pigs showed a very slight reaction at the site of the inoculation but it was so indefinite that the results were regarded as doubtful.

Four days later all 12 guinea pigs were retested with 2 minims of a dilution of equal parts of saline and Koch's old tuberculin by intradermal inoculation, and after twenty-four hours no reactions were observed that could be considered definite. Three days after this inoculation, 9 of the 12 retested guinea pigs showed very marked skin reaction, the remaining 3 showed absolutely no reaction. Five of these guinea pigs, 3 (A, B, C) giving negative and 2 (D and E) positive tests, were selected for special observation.

Guinea Pig A (in cage with C and D) showed no skin reaction after test and was used as a control. Died March 15, 1927. Necropsy revealed slight localized inflammation at site of inoculation, enlarged inguinal glands on left side and organs normal in appearance. Microscopic examination of smears made from organs and glands, stained by Ziehl-Neelson's method, failed to demonstrate any acid-fast bacilli.

Guinea Pig B (in separate cage) showed no skin reaction after first test and on March 7 was inoculated subcutaneously with washed live organisms from a pure culture of *Mycobacterium tuberculosis* (hominis) and on retesting gave positive skin reactions. Died April 22, 1927. Necropsy revealed no abnormal conditions of organs but enlarged inguinal glands on both sides. Microscopic examination of smears made from organs and glands, stained by Ziehl-Neelson's method, demonstrated numerous acid-fast bacilli in all slides.

Guinea Pig C (in cage with A and D) showed no skin reaction after test and on March 7 was inoculated subcutaneously with washed killed organisms from a pure culture of *Mycobacterium tuberculosis* (hominis), (organisms

placed in autoclave and subjected to the heat of flowing steam for one hour), on retesting gave negative skin reaction. Died March 22 1927. Guinea pigs B and C were retested on the same day (eleven days later). Necropsy revealed organs and glands in apparently normal condition. Microscopic examination of smears made from organs and glands, stained by Ziehl-Neelsen method, failed to demonstrate any acid fast bacilli, but adjacent glands showed a fairly typical focus, simulating tuberculosis.

Guinea Pig D (in cage with A and C) showed marbled skin reaction after test and was used as a control. Died March 20 1927. Necropsy revealed organs in apparently normal condition but enlarged inguinal glands on both sides. Microscopic examination of smears made from organs stained by Ziehl-Neelsen method, failed to demonstrate any acid fast bacilli in the organs but showed typical acid fast bacilli in the lymph nodes.

Guinea Pig E (in pen in animal room) showed marbled skin reaction after test and was used as a control. Died March 19, 1927. Necropsy revealed slightly enlarged, but practically normal inguinal glands and organs, with the exception of right lung in apparently normal condition. Right lung at base showed white cheesy area. Microscopic examination of smears made from organs and glands stained by Ziehl-Neelsen method, failed to demonstrate any acid fast bacilli in organs or glands with the exception of smear made from right lung. This slide showed typical acid fast bacilli.

Twelve additional young guinea pigs supposedly healthy weighing about 250 gm and bought from a different concern were placed on the floor of the animal room under the same conditions as the previous lot. They were allowed to remain there for a period of four days for observation.

March 30, the 12 guinea pigs were prepared in a similar manner to the first lot and tested with 2 minims of a solution of equal parts of Koch's old tuberculin and saline by intradermal inoculation. After twenty-four hours 4 of these guinea pigs showed a slight redness at site of inoculation which might be interpreted as the beginning of a reaction but all 4 were doubtful. The other 8 were frankly negative. Forty-eight hours after inoculation 3 of these doubtful guinea pigs developed a positive skin reaction and the other 9 were negative. Some of these animals were killed by the dog in the animal room, the rest were used for subsequent experiments.

DISCUSSION

It will be observed that 75 per cent of the first lot and 25 per cent of the second lot gave positive skin reactions. While the results of the second lot confirmed those of the first, we were unable to follow the same procedure because they were used for subsequent experiments, results of which will be published at some future time. We must summarize the results from the originally selected lot of guinea pigs, however limited in number for we feel that it is of sufficient interest to justify publication. The injection of killed organisms did not produce any skin sensitiveness to tuberculin in guinea pig C, although Lange³ states a transient hypersensitiveness can sometimes be obtained from the injection of a large amount of killed culture. The results in guinea pigs D and E were most interesting and on the findings

of these animals, in addition to the above experiments, we feel that we may emphasize the necessity of testing guinea pigs with tuberculin to exclude a tuberculous focus before using them for laboratory diagnosis or experiments

Guinea pigs D and E both showed marked skin reactions after tests and were kept under observation and used as controls. On necropsy these animals revealed typical tuberculous lesions in which the acid-fast bacilli were found. Guinea pig B, giving negative skin reaction, was inoculated with washed live organisms and subsequently gave a positive skin reaction, and showed the same pathology on necropsy as guinea pig D which gave a positive skin reaction on all tests and was used as a control, emphasizing that there was a pre-existing focus in A similar to that produced in B by artificial inoculation.

Furthermore, necropsies were performed on some of the guinea pigs that had previously given a positive tuberculin test but died for reasons unknown, and in not one instance did we fail to find evidence of tuberculosis.

CONCLUSIONS

- 1 Tuberculosis is quite prevalent in apparently normal guinea pigs
- 2 An intracutaneous tuberculin test reveals the presence of a tuberculous lesion in guinea pigs
- 3 Guinea pigs injected with dead tubercle bacilli did not develop any sensitivity to tuberculin
- 4 Guinea pigs may have a preexisting tuberculous focus similar to that induced by inoculation
- 5 Guinea pigs of uncertain history should be tested with tuberculin before being employed for the inoculation diagnosis of tuberculosis and other laboratory experiments relative to tuberculosis

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PLASMA PROTEIN IN RELATION TO SUSPENSION STABILITY OF ERYTHROCYTES AND PRECIPITATION OF SERUM PROTEIN WITH ALUMINUM SULPHATE*

By L. R. JONES, PH.D., St. Louis, Mo

THE sedimentation rate of erythrocytes and numerous serum precipitation (flocculation and ring) tests have long been used for determining the presence of infection in the human subject. Numerous experiments indicate that the etiology of an increased sedimentation rate of erythrocytes is invested in the plasma rather than the cells, since upon transferring cells of normal blood to plasma obtained from a diseased individual, the phenomenon of an increased sedimentation rate is observed. However the rate of sedimentation is within normal limits if cells from a diseased individual are added to plasma from a normal subject.

The observation that protein can be precipitated from serum of the diseased with smaller amounts of the precipitating agent than is possible with the serum of the normal person, has been variously explained as being due to an alteration in the distribution of the serum proteins as might be observed with relative or absolute increases or decreases in the several fractions of serum protein, to the presence of certain lipids and to variation in content of electrolytes. This investigation was undertaken in an effort to determine whether the velocity of sedimentation of erythrocytes, and the precipitability of serum protein as determined by the addition of aluminum sulphate, could be correlated, with quantitative relationship of the various plasma proteins.

That an increased rate of sedimentation of the erythrocytes obtains in the blood of the tuberculous and that the sera of such individuals exhibits an increased precipitability with aluminum sulphate has been previously reported by the author^{1, 2}.

This investigation involved the estimation of fibrin, globulin, and albumin of plasma, sedimentation rate of erythrocytes in citrated blood, and the quantitative relationship of aluminum sulphate to the precipitation of serum protein of human blood. Since normal values for these blood properties must be known before it is possible to make any inference regarding pathologic conditions, examinations were made upon the blood of 20 normal individuals to establish a basis for comparison. In addition this report incorporates the values for these blood properties as observed in the blood of 20 individuals suffering from such conditions as hypertension, carcinoma, acute infections, ascites, and tuberculosis.

From the Department of Bacteriology and Hygiene, St. Louis University School of Medicine.

Received for publication July 9, 1929.

METHODS EMPLOYED

Blood specimens were collected from an arm vein (without stasis) after a fasting period of fourteen hours. A portion of the specimen was oxalated, the clear plasma separated, and fractionation of the protein was promptly undertaken. Fibrin was separated by recalcifying diluted plasma, and collected on a small glass rod. Globulin was coagulated with 15 molar sodium sulphate and removed by filtration, the nitrogen of the filtrate representing that contained in the albumin and nonprotein constituents. Nonprotein-nitrogen was determined as contained in the tungstic acid filtrate. These fractions were quantitatively determined by calculation from the amount of nitrogen recovered after Kjeldahl digestion.

In determining the erythrocyte sedimentation rate (ESR) three parts of blood were mixed with one part of 3.8 per cent sodium citrate solution. Serologic pipettes of 1 c.c. capacity, delivery type (1 c.c. volume contained in a lineal distance of 135 mm. from 0 to 10 graduation marks) were filled with the citrated blood by aspiration and the delivery end sealed with beeswax. The pipettes were then set aside in a vertical position at room temperature (approximately 20° C.) and readings made at one-half, one, and two hour intervals, the upper surface of the corpuscle layer serving as a guide from which to record the descending cell column, using the pipette graduations as a convenient scale for recording the extent of fall at the various time intervals. The cell volume of the blood was determined with the Van Allen hematocrit tube. The volume of the individual corpuscle was determined according to the following relation:

$$\text{Volume of corpuscle c mm} = \frac{\text{Cell volume per cent}}{\text{Number cells per c mm}}$$

For the flocculation test a portion of the blood specimen was set aside for an hour and a half to insure complete clotting after which the serum was separated by centrifuging, and collected. Various amounts of aluminum sulphate (ranging in concentration from 0.02 to 0.12 per cent) contained in a unit volume of 1 c.c. were added to 0.2 c.c. of the blood serum in small test tubes. The serum and reagent were carefully mixed by inverting the tube three or four times. It was observed that more extensive shaking affected only the character of the precipitate as indicated by the formation of somewhat larger flocculi and even very extensive shaking of the mixture was without result upon the incidence of precipitation. The tubes were set aside at room temperature (approximately 20° C.) for one and one-half hours, prior to the reading. Preliminary experiment revealed that incubation at a higher or lower temperature, as 37° C. or 10° C., affected the degree of precipitation quite irregularly, consequently all tests were made at or near 20° C., as a standard procedure. In reading the tubes, a heavy flocculent precipitate that settled out, leaving a clear supernatant fluid was recorded as "xxxx" while a very small amount of flocculent precipitate was recorded as a "x" reaction. Precipitates intermediate in amount were recorded as "xxx" and "xx" reactions according to the amount and density. Uniformity in readings was soon attained after a moderate amount of experience in examining the tubes. Atypical types of flocculation were only rarely observed.

Sera eventually yielding a heavy precipitate usually gave a prompt turbidity upon mixing the serum and reagent. In some instances a definite turbidity appeared within as short an interval as fifteen minutes. However it did not seem feasible to evaluate the precipitability of the serum by the length of the incubation period required for the appearance of a turbidity or definite precipitate, as has been suggested recently by Bodon,⁴ as many of the sera yielding heavy precipitates within an incubation period of one hour and a half did not exhibit a definite turbidity immediately upon mixing serum and reagent.

Protein determinations herein reported are based upon plasma rather than upon serum, which was used in all of the precipitation experiments. Concentration of the globulin and albumin fractions in plasma is not entirely comparable to the concentration obtaining in serum. However the possible discrepancy as determined by numerous experiments is not of great magnitude.

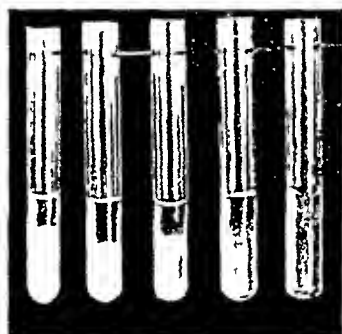


Fig 1—Precipitation of serum protein with aluminum sulphate. Reading from left to right
xxxx xxx xx x and negative

The trend of significant alteration in the protein quotient of serum is readily established by determination of the globulin and albumin contained in the plasma.

EXPERIMENTAL

Since the interpretation of any alteration in these blood properties as observed in the diseased individual, must be predicated upon a conception of values obtaining in the normal state, the results obtained in the examination of the venous blood of 20 normal men are given in Table I.

The values for total protein and protein fractions as obtained in this study are not at great variance with the commonly accepted standards for individuals of this class, although in the results observed wide variation may exist between individuals. Sedimentation readings of 0.13 and 0.27 were the maximum values observed for the one and two hour periods respectively. Hence it may be assumed that values significantly greater than these in this class of individuals would be indicative of an accelerated ESR. Variability in the sedimentation rate is not readily correlated with variation in the quan

TABLE II
HUMAN BLOOD IN MORBID CONDITIONS

CASE	ML	FD	JG	EDM	VO	GM	MG	GB	DD	MF	MH	POZ	SGH	PMI	FW
SEX	M	F	M	M	M	F	M	M	F	F	F	M	M	M	F
AGE—YEARS	71	48	70	59	62	58	50	45	58	57	16	47	45	23	42
WEIGHT—POUNDS	175	90	130	140	150	115	150	130	125	200	120	140	150	125	140
Plasma—100 cc	GANCINOMA	RECTAL ABSCESS	MYOCARDIAL INFARCTION	F CIRRHOSIS	SYMPTOMATIC PURPURA	LYMPH ABSCESS	CHOLEMIA	HYPERTENSION NEPHRITIS	CHRONIC PLEURIS	HYPERARTERIO SCLEROSIS	LIPIDEMIA	PULMONARY TUBERCULOSIS	SULPHURIO OXIDEMIA	PULMONARY TUBERCULOSIS	HYPERARTERIO SCLEROSIS
Total proteins gm	6.46	8.71	6.48	6.17	6.65	7.00	6.63	6.81	6.01	8.69	8.93	7.07	7.78	7.54	7.33
Fibrin gm	0.44	0.66	0.27	0.27	0.41	0.79	0.31	0.32	0.20	0.61	0.36	0.54	0.45	0.41	0.29
Glob gm	2.95	3.99	2.50	2.91	2.90	3.04	2.54	2.68	1.87	3.18	4.13	2.55	2.38	2.27	1.54
Alb gm	3.07	4.06	2.86	2.89	3.34	4.07	3.78	3.81	2.83	4.90	4.44	4.63	4.95	4.90	5.50
Prot Quot. Albumin-Globulin	1.0	1.0	1.0	1.0	1.1	1.3	1.4	1.4	1.5	1.5	1.7	1.7	2.0	2.1	3.5
Erythrocytes	0.40	0.56	0.01	0.16	0.50	0.38	0.27	0.02	0.08	0.07	0.06	0.40	0.02	0.15	0.01
Sedimentation Rate 30 mm	0.54	0.65	0.02	0.43	0.73	0.60	0.39	0.12	0.13	0.20	0.20	0.53	0.04	0.23	0.03
1 hr	0.02	0.71	0.08	0.45	0.83	0.69	0.53	0.18	0.21	0.32	0.50	0.57	0.09	0.39	0.10
2 hr	3.72	3.67	5.30	3.47	1.24	4.30	4.00	5.59	4.54	4.83	2.90	4.10	6.08	5.30	4.46
Erythrocytes per c mm x 10 ⁶	38.5	33.0	58.0	40.0	14.0	40.0	40.5	64.0	59.0	58.5	25.0	38.0	48.0	52.0	49.0
Cell Volume Hematocrit per cent	10.0	8.9	10.9	13.0	11.0	9.3	10.0	11.0	12.0	12.0	8.6	9.7	7.8	9.8	10.9
Volume of corpuscle c mm x 10 ⁻³															
Serum precipitation with aluminum sulphate	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.10						
Per cent	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.10						

titative distribution of plasma proteins or in the concentration of erythrocytes in the blood

It is significant that precipitability of the blood serum is not correlated with the ratio of albumin to globulin, as contained in the plasma. Considerable variation in precipitability is to be noted among these normal sera. However in no instance did precipitation occur with 0.05 per cent of aluminum sulphate in the reagent, an amount designated by Matefy⁶ as indicating an abnormal serum lability of tuberculous individuals.

Further study of the relationship existing between the blood properties that constitute the subject of this investigation was made upon the blood of a small number of human subjects, individually presenting a fairly well-defined type of pathology. No attempt was made to determine the characteristic blood alteration obtaining in any given disease as the number of cases available for study was inadequate for such a purpose. The various clinical diagnoses and the results of these examinations are listed in Table II.

These findings, in many instances, present a departure from the established limits of normal variation that is of considerable magnitude.

DISCUSSION

The data indicate that sedimentability of the blood is not correlated with significant variation in the size of the individual corpuscle. In many instances an increased ESR is observed in blood having a low content of red blood cells so that an anemia per se may upon occasion be a determining factor in this phenomenon. It is apparent that the concentration of erythrocytes in the blood must be considered in evaluating the significance of an increased ESR, as the concentration may have an inverse relationship to the speed of sedimentation.

In the blood of normal individuals sedimentability of the erythrocytes is a variable property and the variation observed between normal individuals is not to be explained upon the basis of quantitative distribution of the respective plasma proteins. (In repeated examinations of the blood of some of these normal individuals, covering a period of several months, the magnitude of periodic variation approached or was equal to that observed between various individuals.)

An increased ESR was observed in the presence of various morbid conditions and in most instances there was also observed either an increase in fibrinogen or a decrease in the ratio of albumin to globulin. Therefore, it seems possible that these factors may independently or jointly be determinative of the ESR.

Minimal concentration of aluminum sulphate effecting protein precipitation in the serum of the normal human subject was determined. Precipitation with a concentration of less than 0.06 per cent may be considered indicative of increased precipitability of serum constituents. Precipitation occurred in all of the normal sera at a concentration of 0.09 per cent or less. In the presence of various diseases the marked increase in serum precipitability is not correlated with alteration in the ratio of globulin to albumin as contained in the plasma.

In general, the addition of an electrolyte, such as aluminum sulphate to serum, yields a precipitate (often referred to as a metallic albuminate) which is perhaps not a true combination, but a double salt or loose absorption compound of the protein with the salt. This reaction may be considered quasi reversible in that dilution with water or removal of the salt by dialysis does not restore the changed protein. However, such precipitates are soluble in an excess of the salt solution. Although hydrolysis occurs in an aqueous solution, rendering the solution acid perhaps the effectiveness of this salt as a precipitating agent is explained on the basis that electro negative colloids are precipitated by the cation and in dilute solution the flocculation effected is related to or a function of its valence.

It is noted that blood exhibiting the phenomenon of an increased ESR quite regularly exhibits in addition the property of increased precipitability of the serum. This correlation is observed regularly except in conditions wherein the extreme anemia per se may serve to explain the accelerated ESR.

SUMMARY

1 Increased sedimentability of the erythrocytes in the blood of the diseased is often associated with a shift in plasma protein toward the more labile fibrinogen and globulin fractions

2 Increased precipitability of serum protein as determined with aluminum sulphate, is observed in the presence of various morbid conditions and may or may not bear relation to the quantitative distribution of plasma proteins

3 A correlation between sedimentability of red blood cells and precipitability of serum protein is quite regularly observed

4 Diagnostic value of the aluminum sulphate serum flocculation test, may be enhanced by titration of the serum with graded amounts of the reagent, as herein accomplished

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CARBOHYDRATE METABOLISM IN ACROMEGALY*

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THE most exhaustive work on this subject is by Davidoff and Cushing¹ These authors review 100 cases which they have studied and discuss their conception of the mechanism involved in the disturbance of the carbohydrate metabolism The subject is of importance not only per se, but also because there are certain fundamental principles involved, which have a general application

Hansemann² found approximately 12 per cent among 97 reported cases of patients with acromegaly to be suffering from diabetes mellitus, Hinsdale³ found 11 per cent in 130 cases, Borchardt,⁴ on the other hand, found 35.5 per cent of 176 cases recorded up to 1908 The variability of these percentages doubtless depends a great deal upon what the compiler of the statistics has been willing to accept as "diabetes mellitus" Davidoff and Cushing in their series of 100 personally observed cases of acromegaly found one out of four to have glycosuria, and one out of eight to have clinically outspoken diabetes mellitus The question is this "Given a case of glycosuria, what criteria shall one employ to determine its nature?"

It is not within the scope of this paper to discuss the various theories advanced for the explanation of the disturbance of the carbohydrate metabolism in acromegaly It seems quite certain that to elucidate this problem the relationship of the pancreas to the hypophysis must be determined

Davidoff and Cushing state the following "It is conceivable that the oversecretion of the hypophyseal substance may indirectly produce glycosuria in one of two ways (1) by neutralizing the secretion of the islet tissue in circulation, thereby causing compensatory hypertrophy with ultimate exhaustion of the cells of Langerhans, leading to the degenerative changes with which we are familiar, or (2) by actually checking the secretory function of the islets which might possibly lead to no histopathologic changes However this may be, it seems wholly improbable that the diabetes in acromegaly is due to an independent and coincidental lesion, functional or otherwise, of the pancreatic islets"

It has been conclusively shown by Evans⁵ and his co-workers that acromegaly is an expression of the hyperfunction of the pituitary gland, and more specifically, an oversecretion of the acidophilic cells of the pars anterior

J H Burn⁶ has done work to demonstrate an antagonism between insulin and pituitrin He showed that pituitrin (posterior lobe extract) definitely neutralizes the power of insulin to lower blood sugar, this was not true of

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anterior lobe extracts. He considers this antagonism to be in the nature of a chemical binding of insulin by pituitin. Corroborative work was done by Davidoff and Cushing¹. In three of their patients with acromegalic diabetes who had been shown to respond normally to insulin, 20 units of insulin combined with 1 c.c. of solution of pituitary (Pituitrin, P. D. & Co.) were injected. The expected fall in blood sugar did not occur.

An interesting point is this: Why should posterior lobe extracts be those to counteract insulin, when, as far as we know, hyperpituitarism is an anterior lobe disorder? Davidoff and Cushing state that possibly the posterior and anterior lobes have interdependent rather than the quite separate functions generally ascribed to them.

It was stated above that Davidoff and Cushing found glycosuria in one fourth of their cases, but considered diabetes mellitus to be present only in one eighth. They also found in a study of seven cases which had no glycosuria, that all had a blood sugar curve higher than normal. In other words, all cases of acromegaly have a disturbance of carbohydrate metabolism. If the mechanism described by Davidoff and Cushing is the sole one operating, then all cases of acromegaly should have diabetes mellitus, or none should have it.

In discussing carbohydrate metabolism, two phases must be considered: (1) the storage of carbohydrates (glycogenesis and glycogenolysis), and (2) the oxidation of glucose. Thus the administration of adrenalin favors glycogenolysis, with consequent hyperglycemia and glycosuria, and an increase in the oxidation of glucose. Insulin favors glycogenesis and an increase in the oxidation of glucose. It is evident that considered separately, insulin and adrenalin have opposite actions as far as the storage of carbohydrates is concerned, but act similarly on the oxidation mechanism.

It would be well to digress for a moment and consider the following point which is closely related to this subject. It is stated that the administration of adrenalin produces a diminution in glucose tolerance, for if adrenalin is given to a susceptible individual who has received 100 gm. of glucose, he will exhibit a glycosuria. The blood sugar curve will resemble that of a mild diabetic. The respiratory quotient during this period, however, will rise from a fasting level of, let us say 0.80, to almost 1. The amount of glucose oxidized per unit time is greater than in a normal individual for there is an increase in the amount of oxygen consumed in the individual receiving adrenalin. A mild diabetic given 100 gm. of glucose, may exhibit the same blood sugar curve, and the same amount of glycosuria, his fasting respiratory quotient may also be 0.80, but will rise only moderately, let us say to 0.86. Surely it cannot be said that both have a diminished tolerance for glucose. From the teleological standpoint the important phase of carbohydrate metabolism is oxidation. It was shown above that more glucose is oxidized per unit time by an individual receiving adrenalin than by a normal one. The glycosuria is simply incidental to the hyperglycemia. To state then, that the administration of adrenalin engenders a diminution in glucose tolerance is misleading. A deficiency in glucose tolerance should be considered as existing only when there is an impairment in both the ability to oxidize and store glucose.

With this as an introduction, we may now proceed to a discussion of the influence of pituitrin on carbohydrate metabolism. It was stated above that Davidoff and Cushing¹ and Burn⁶ found that pituitrin neutralizes the effect of insulin on blood-sugar concentration. The experiments by Cammidge and Howard⁷ on the interrelation of insulin and other gland extracts on metabolism are of great value. They showed that the injection of insulin produces an increase in the respiratory quotient in fasting animals, which can be prevented by the administration of pituitrin. Their work on fed animals, however, is of far greater importance. They determined that white rats, after an abstinence of twenty-four hours from food, gave a respiratory quotient of 0.77. After feeding, the respiratory quotient was found to have increased to 0.91. The increase in the respiratory quotient is to some extent due to the conversion of carbohydrate to fat, but in greater part it is the result of the oxidation of glucose. It is logical to assume that this rise in respiratory quotient is a result of the secretion of insulin by the pancreas, for were these animals to be pancreatectomized, the respiratory quotient would not rise on feeding, and marked hyperglycemia and glycosuria would also appear. They found that the injection of $\frac{1}{4}$ c.c. of pituitrin caused a glycosuria in all animals, *but the respiratory quotient remained 0.91*. In other words pituitrin interfered with the action of insulin on the blood-sugar level, as is evidenced by its producing hyperglycemia and glycosuria, but it in no way interfered with the oxidation of glucose. This fact is of fundamental importance. It contradicts the statement of Burn⁶ and of Davidoff and Cushing¹ that pituitrin completely nullifies the action of insulin.

We believe that we are justified in drawing the following conclusions. If an adequate amount of insulin is secreted by the islets, there is no known substance, as far as we are aware, which will interfere with its power to cause the oxidation of glucose. The failure of the respiratory quotient during fasting to rise markedly after the ingestion of large quantities of glucose is evidence of a deficiency of the Islands of Langerhans. Conversely the presence of a respiratory quotient approaching 1 after the administration of large quantities of glucose, is absolute evidence of normally functioning islets, regardless of the extent of hyperglycemia and glycosuria.*

With these facts at hand we may now consider carbohydrate metabolism in acromegaly. Practically all cases of acromegaly have a blood-sugar tolerance curve above normal (Davidoff and Cushing). All other factors being equal the presence of glycosuria will depend simply on the extent of the hyperglycemia. Now the question is: Which of these cases have diabetes mellitus? This can be conclusively answered by studying the respiratory quotient after giving large quantities of glucose. If the respiratory quotient approaches 1 the functional activity of the islets may be considered normal, if the respiratory quotient is much less than 1, then we must conclude that there is an inadequate secretion of insulin by the Islands of Langerhans, this constitutes diabetes mellitus.

In the nondiabetic cases the hyperglycemia will be caused by the excessive

*This does not include phlorizin diabetes where the respiratory quotient rises only slightly after the administration of glucose. This is a result not of the inability of the organism to oxidize glucose but of the rapid elimination of glucose through the kidney.

pituitary secretion only, whereas in the diabetes two factors will be operating to produce an increase in the blood sugar concentration, the increased pituitary secretion and the diminished insulin secretion

We will now present a patient with acromegaly who came to our diabetic clinic because of the presence of glycosuria. A glucose tolerance test was performed, 150 gm of glucose were given (Table I). The blood sugar content was determined by the Kramer Gittelman⁸ micro method (modification of Folin Wu) in which blood is obtained by pricking the finger tip. It has been shown by Foster⁹ that this blood is equivalent to arterial blood. This is

BLOOD-SUGAR CURVE

BLOOD SUGAR MG/100 CC	FASTING LEVEL	45 MINUTES PC*	TWO HOURS PC
	202	380	416

RESPIRATORY QUOTIENT CURVE

FASTING LEVEL	1½ HR PC	2½ HR PC
0.78	0.78	0.82

PC—After ingestion of 150 grams of glucose.

not significant as far as this work is concerned. The respiratory quotients were determined by Bailey's method (Tissot spirometer and Henderson Haldane gas burette).

This patient is suffering from diabetes mellitus

It is very interesting to note that although there may be marked glycosuria in acromegalic diabetes, ketosis is often not present or if present only of slight degree. This may be explained as follows. As stated above the glycosuria in acromegalic diabetes is due to the sum of two factors: (1) the excessive pituitary secretion and (2) the deficiency in insulin secretion. Thus although there may be only slight reduction in the amount of insulin secreted, the glycosuria nevertheless will be marked. Since the oxidation of glucose in this case is only moderately affected, ketosis will not result or be only slight in amount.

It is evident that the work on which our conception is based is meager in amount. It fits in well, however, with all the phases of the question. We believe that continued study of more cases of acromegaly along the lines laid down by us will lead to a complete elucidation of the problem.

SUMMARY AND CONCLUSION

Although acromegaly is caused by a diseased state of the anterior lobe of the pituitary gland, it is the secretion of the posterior lobe which has been shown experimentally to affect carbohydrate metabolism. Davidoff and Cushing state: "It possibly may come to be shown that the posterior and anterior lobes have interdependent rather than the quite separate functions generally attributed to them."

The disturbance in carbohydrate metabolism in acromegaly may be incidental to it or to concomitant diabetes mellitus.

Of cardinal importance is the fact that although pituitrin neutralizes the effect of insulin on blood-sugar concentration, it does not affect the action of insulin on the oxidation mechanism of glucose

This fact may be utilized in determining the nature of the carbohydrate disturbance. If the respiratory quotient, after the administration of a large quantity of glucose fails to approach 1, diabetes mellitus is present, if the respiratory quotient does approach unity, the glycosuria is symptomatic regardless of its degree

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STUDIES ON FAT METABOLISM*

I FAT TOLERANCE IN OBESITY

A PRELIMINARY STUDY

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SOME forms of obesity have been generally considered as due to some disturbance of fat metabolism, a distinction being made between exogenous obesity, due to overfeeding and inactivity, and endogenous obesity, due to a variety of abnormal physiologic or pathologic functions. Newburgh¹ has recently presented evidence to support the view that the obese subjects, irrespective of disease or constitutional tendency, are obese because they take in more than is used up, or the disease with which it is associated causes obesity by increasing the appetite or decreasing the activity of a formerly active individual. Much study is necessary to prove the correctness or falsity of these views.

Since the study of sugar tolerance and sugar metabolism in diabetes mellitus has proved to be of such value, and since fat metabolism and obesity are intimately related to sugar tolerance and sugar metabolism, and since obesity is so closely associated with the disease of certain organs, studies on fat tolerance in obesity might prove to be of great value.

Our ideas on how fat gets into the blood and how it is oxidized are fairly clear, but only a little is known concerning the intermediate stages of fat metabolism. Bloor has found on the ingestion of a fat meal that the total fatty acids in plasma and corpuscles increase, that the lecithin in the corpuscles increases without much change in the plasma and that no definite change occurs in the cholesterol. Cowie and Hoag² administered fat in the form of cream to normal children and adults. They found that in five children the maximum lipid content of the blood was reached in from five to seven hours and in three adults at the sixth hour. When the fat was given with a large amount of sugar, they found that the highest lipid content was reached at the second hour. The relation of carbohydrates to the fat in the diet of the diabetic patient is thoroughly discussed by Joslin,³ which shows that the two are related in some way. The results of Rabinowitch⁴ show that insulin has a marked effect in reducing the blood lipids in diabetes, and according to Chauffard⁵ and Labbe⁶ insulin affects fat metabolism independently of its effect on carbohydrate metabolism.

Such observations have caused us to ask ourselves the following questions: Is the alimentary lipemia of an obese subject different from that of a normal subject? Is it possible to differentiate between certain types of obesity on the basis of the alimentary lipemia curve? What factors, and especially do

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hormonal factors, play a rôle in the regulation of the movements of fat in the obese subject? The work presented in this paper is a preliminary attempt to find an answer for these questions. It is possible that a study of obesity from this viewpoint may prove to be of more value than has been the study of basal metabolism, the respiratory quotient and the specific dynamic action of foods.

METHODS

Bloor's method⁷ was used for the determination of the total plasma lipoids, cholesterol and fatty acids. Cubital vein blood was drawn and the citrated plasma used. Three samples of the petroleum ether extract were used each time. The results on these samples never varied more than 3 per cent.

The blood fat content of normal and obese subjects was determined fifteen hours (overnight) after the last meal. Marked cases of obesity were selected for this purpose, the average overweight being 95 pounds, and their physical examination and complaint other than obesity being negative.

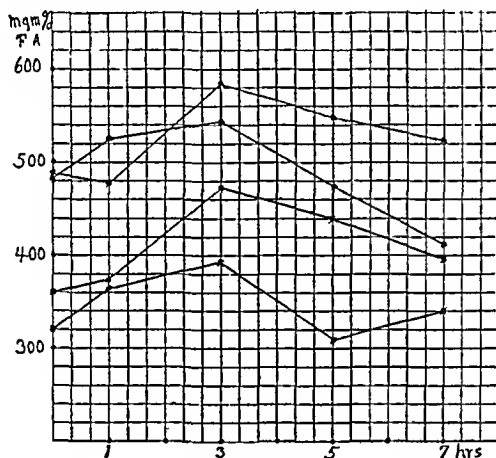


Fig 1—Normal fat tolerance curves

The alimentary fat tolerance test was performed as follows: (1) blood was taken at 9 00 A M, 15 hours after the last meal, (2) a pint of 20 per cent cream was then given the patient which was followed by approximately 500 cc of water, (3) blood samples were drawn at one, three, five, and seven hours after the meal of cream, no food nor water being allowed until after the seven hour blood sample was drawn.

RESULTS

The total fatty acid content of the blood plasma of fifteen normal subjects taken in the morning fifteen hours after the last meal averaged 0.373 per cent. The maximum content was 0.488 per cent, and the minimum was 0.255 per cent. These results compare favorably with those of Bloor whose average was 0.370 per cent and with those of McClure and Huntsinger⁸ whose average was 0.364 per cent.

The average cholesterol content of the blood plasma of this normal group was 0.132 per cent, which is definitely lower than Bloor's average. The maximum value in this group was 0.300 per cent, the minimum, 0.072 per cent.

TABLE I
FAT TOLERANCE IN NORMAL PERSONS

NAME OF CASE	Before Fat Meal	GA	RA	BL	O C	RY	MA	HC	CA
Total Lipids Mg in 100 cc Blood Plasma	After Fat Meal	481	304	625	41	480	411	504	64
	1 Hour	489	388	670	419	506	400	391	60
	3 Hours	509	397	697	477	599	491	641	738
	5 Hours	536	321	616	508	578	414	560	682
Cholesterol Mg in 100 cc Blood Plasma	7 Hours			32	501	530	439	588	666
	Before Fat Meal	111	144	140	39	10	2	120	104
	1 Hour	111	10	151	119	109	90	129	144
	3 Hours	120	133	131	97	123	96	141	154
Total Fatty Acids Mg in 100 cc Blood Plasma	5 Hours	120	140	144	103	134	10	120	133
	7 Hours			140	101	112	96	132	138
	Before Fat Meal	370	360	48	313	300	32	384	489
	1 Hour	378	436	34	330	377	36	46	470
Maximum Increase of Fatty Acids	3 Hours	389	404	540	380	410	395	500	584
	5 Hours	416	381	472	40	440	312	440	549
	7 Hours			412	400	418	343	456	528
	In Per Cent of Initial Value	12	28	12	23	32	23	30	20
	In mg per 100 cc Plasma	46	104	61	92	116	73	110	96

The total fatty acid content of the blood plasma of twenty obese subjects taken in the morning, fifteen hours after the last meal, averaged 0.435 per cent. The maximum value was 0.691 per cent and the minimum 0.225 per cent. The average cholesterol content of the blood plasma of this obese group was 0.128 per cent.

The alimentary fat tolerance tests were performed on 8 normal and eighteen obese subjects.

In the normal subjects (Table I and Fig. 1) the fatty acid content usually shows an increase within one hour after the meal of cream, the peak of the increase occurring from three to five hours after the meal. Seven hours after the meal the fatty acid content was usually less than the five hour content. In these normal subjects the maximum increase after the fat meal was 116 mg. and the minimum 46 mg. per 100 c.c. of blood. Expressed in percentage of the initial fatty acid value the maximum increase was 32 per cent and the minimum 12 per cent.

The cholesterol content of the blood plasma showed no definite or uniform change in these normal subjects nor in the obese subjects, and for this reason the cholesterol values only appear in Table I. Obviously the change in total lipin was due chiefly to the change in fatty acid.

In Fig. 1 the results on subjects Ca, Bl, Ma, and Ry are shown in the form of a curve. They resemble in a general way a sugar tolerance curve, except for the time factor.

An analysis of the results of the fat tolerance tests in the 18 obese subjects reveals the interesting fact that they can be divided into three groups. Group I (7 cases) in which the fat tolerance curve was like that of normal subjects, Group II (5 cases) in which the fat tolerance might be called "high", and Group III (6 cases) in which the fat tolerance might be called "low".

Table II shows the results on seven cases in which the results of the fat tolerance test are within normal variations, the increase in fatty acids varying from 48 to 106 mg. or expressed in percentage, an increase varying from 16 to 35 per cent.

TABLE II
FAT TOLERANCE IN OBESITY (GROUP I)

Name of Case	TOTAL FATTY ACIDS MG IN 100 C.C. BLOOD PLASMA					MAXIMUM INCREASE OF FATTY ACIDS	
	Before Fat Meal	After Fat Meal				In Per Cent of Initial Value	In mg Per 100 c.c Plasma
		1 Hour	3 Hours	5 Hours	7 Hours		
Al	296	353	344	328	337	18	48
G ₁	457	459	539	535	486	16	82
La	391	398	470	352	333	20	79
Su	402	384	452	505	413	25	103
My	465	540	571	501	477	23	106
Z ₁	333	259	416	361		24	83
Ka	278	315	329	377		35	99

Table III shows the results on 5 cases in which the results of the fat tolerance test indicate that the tolerance of the patient for fat is high. Three of these 5 obese subjects after ingesting the fat meal showed a decrease in blood

plasma fatty acids varying from 66 to 132 mg, one, a slight increase the first hour, which was followed by a decrease, the other case showed a maximum increase of 23 mg or 6 per cent Fig 2 shows the curves on 3 of the cases

Table IV shows the results on the six cases in which the fat tolerance of the subject might be termed "low" The increase in the blood plasma fatty acids

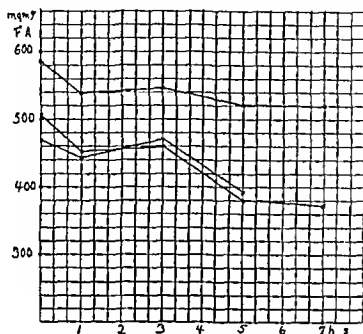


Fig 2—High fat tolerance curves

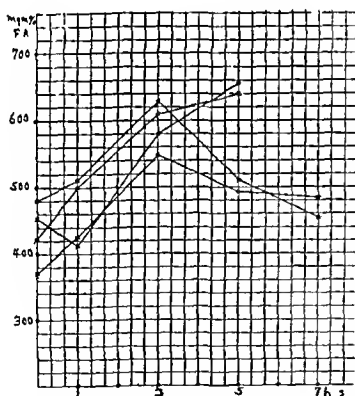


Fig 3—Low fat tolerance curves

varied from 141 to 222 mg or from 31 to 60 per cent, which is greater than that of the 8 normal subjects and 7 of the obese subjects Fig 3 shows the curves on 4 of the cases

These fat tolerance results suggested that the sugar tolerance of these patients be determined So in 17 of the 18 cases sugar tolerance tests were made 175 gm of glucose per kg body weight was given by mouth, the blood sugar and urine being examined before and at one two and three hours after

TABLE III
HIGH FAT TOLERANCE IN OBESITY (GROUP II)

Name of Case	TOTAL FATTY ACIDS MG IN 100 CC BLOOD PLASMA					MAXIMUM CHANGE OF FATTY ACIDS	
	Before Fat Meal	After Fat Meal				In Per Cent of Initial Value	In mg Per 100 cc Plasma
		1 Hour	3 Hours	5 Hours	7 Hours		
Sc	585	541	555	527	519	-11	- 66
Co	470	447	470	393		-16	- 77
Wa	509	449	466	388	377	-26	-132
Gr	691	719	567	653	631	+ 4	+ 28
						-18	-124
Ha	386	409	401	397	342	+ 6	+ 23

In the case in which the sugar tolerance was low, the test was repeated with 100 gm of glucose, regardless of the body weight. The sugar tolerance was considered "low" when the blood sugar mounted to 250 mg or more and was associated with glycosuria. The sugar tolerance was considered "high" when the blood sugar did not rise above 150 mg and the initial blood sugar level was resumed at the end of three hours. The sugar tolerance between these extremes was called average. The correlation of the fat and sugar tolerance results is shown in Table V.

TABLE IV
LOW FAT TOLERANCE IN OBESITY (GROUP III)

Name of Case	TOTAL FATTY ACIDS MG IN 100 CC BLOOD PLASMA					MAXIMUM INCREASE OF FATTY ACIDS	
	Before Fat Meal	After Fat Meal				In Per Cent of Initial Value	In mg Per 100 cc Plasma
		1 Hour	3 Hours	5 Hours	7 Hours		
Go	452	408	578	655		60	203
Fa	422	500	611	640		51	222
McG	476	519	622	583		31	156
Kae	399	426	525	540	525	35	141
Di	372	426	548	492	483	47	176
Be	480	509	634	514	451	32	154

TABLE V
RELATION OF FAT TOLERANCE TO SUGAR TOLERANCE IN OBLSE SUBJECTS

	FAT TOLERANCE			SUGAR TOLERANCE		
High	Wa	Co	Sc	Wa		
	Ha	Gr		Wa		
Average	Li	Al		Li	Co	Be
	Su	Kam		Su	Gr	
	My			My		
	Za			Za		
Low	Go		Be	Go	Al	Sc
	Fa			Fa	Kam	
	McG			McG		
	Kae			Kae		
	Di			Di		

The results shown in Table V demonstrate quite an interesting and striking correlation between the fat and sugar tolerance of obese subjects. Out of the 17 cases there is only one case in which the correlation is contradictory, namely, subject Sc who shows a high fat tolerance and a low sugar tolerance.

A case from the "high" tolerance group and the "low" tolerance groups will be briefly reviewed

1 *Case of High Sugar Tolerance and High Fat Tolerance*—Mrs Wa, twenty seven years old, married, has two children Present weight 298 pounds, height 66 inches, 137 pounds overweight Patient is a member of a stout family, she was always much overweight Menses regular, with normal flow Pulse 90 Blood pressure 120/80 B M R—2 per cent Sugar tolerance test, after ingestion of 175 gm glucose per kg body weight, gives the following blood sugar values 80 mg per cent fasting 134 mg per cent after one hour, 151 mg per cent after two hours, 72 mg per cent after three hours Fat tolerance test shows total fatty acids in blood plasma 509 mg per cent fasting, 449 mg per cent after one hour, 466 mg per cent after three hours, 388 mg per cent after five hours, 377 mg per cent after seven hours

2 *Case of Low Sugar Tolerance and Low Fat Tolerance*—Mrs McG, thirty one years old, married has one child Present weight, 298 pounds, height 68 inches, 135 pounds overweight Patient was always overweight, but gained particularly in the last seven years after an operation when both ovaries were removed Pulse 72 Blood pressure 178/104 B M R—5 per cent Sugar tolerance test with 175 gm glucose per kg body weight 104 mg per cent (fasting value), 256 mg per cent after one hour 278 mg per cent after two hours, with 2 per cent sugar in the urine, 256 mg per cent after three hours, with 15 per cent sugar in the urine Test repeated two weeks later with 100 gm glucose 92 mg per cent (fasting value) 206 mg per cent with 0.4 per cent sugar in the urine after one hour 168 mg per cent after two hours, 132 mg per cent after three hours Fat tolerance test 476 mg per cent (fasting value), 519 mg per cent after one hour, 622 mg per cent after three hours, 583 mg per cent after five hours

DISCUSSION

A comparison of the fasting fatty acids of the blood plasma of normal and obese subjects shows that the average fatty acid content of the latter groups is somewhat higher (62 mg) than the average of the normal group The maximum and minimum values in the subjects of both groups, however fall almost within the same limits which detracts from the significance of the fasting blood fat values in any single obese subject

The observation that all obese subjects do not react similarly to the fat meal, but may be divided into three groups, one in which the fat tolerance may be called 'high,' one in which it may be called 'average' and the third in which it may be called 'low,' is highly suggestive and may have a fundamental bearing either on the problem of obesity or on the problem of the relation of obesity to the endocrine glands Considerable speculation and theorization based on observations in the literature and on known facts might be entered upon at this point in our discussion, but because of the preliminary nature of this paper, it will be omitted One point might be emphasized, however namely the close relation which apparently exists between alimentary lipemia and sugar tolerance It can be safely stated that in the presence of a normal liver the insulin output of the pancreas is the most important factor controlling

sugar tolerance The correlation between the fat and sugar tolerance in our subjects indicates that the blood fat is controlled by insulin, in a manner similar to that by which insulin controls blood sugar This view may be expressed as follows Just as insulin promotes the formation of glycogen from the circulating blood sugar, insulin also promotes the deposition of tissue fat from the circulating blood fat

Obviously fat tolerance tests on more obese subjects should be made, and more than one test should be made on each case Also, tests should be made on "constitutionally lean" subjects This problem also lends itself to animal experimentation We are now engaged in performing such experiments

SUMMARY

1 The average fasting fatty acid content of the blood plasma of obese subjects is somewhat higher than that of normal subjects This finding does not have much significance in the individual case because of the variations in different cases

2 The effect of the administration of a pint of 20 per cent cream on the blood plasma lipids of 8 normal and 18 obese subjects was studied No definite change resulted in plasma cholesterol The fatty acid content was increased in the normal subjects and a fairly uniform curve resulted, a maximum content being reached from three to five hours after the meal of cream The results on the 18 obese subjects showed that 7 gave a normal response, and that 11 gave an abnormal response Of these 11 cases, 5 showed a "high" tolerance, the fatty acid content decreasing after the meal, and 6 showed a "low" tolerance, the fatty acid content increasing more than 116 mg or the highest of the normal cases

3 A correlation exists between the "sugar tolerance" and the "fat tolerance" in obese subjects If the sugar tolerance is high, the fat tolerance is high, if the sugar tolerance is low, the fat tolerance is low, if the sugar tolerance is "average," the fat tolerance is "average"

We desire to express our thanks to Dr A C Ivy for his interest and advice

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THE RELATIONSHIP OF POTASSIUM TO THE DEPRESSOR EFFECT OF LIVER EXTRACT*

By RALPH H MAJOR M.D., AND C J WEBER PH.D. KANSAS CITY KANS

GOERNER and Haley in a recent article on the nature of the depressor substance in hepatic extract, state that, "as a result of our investigations on liver extract, we believe that the depressor substance is monopotassium dihydrogen phosphate with possibly traces of other potassium salts aiding this action. It would also seem that the potassium ion is the active depressor agent, since other phosphates fail to cause depression and other potassium salts react positively."

Goerner and Haley obtained from liver extract a batch of crystals which, when purified, proved to be crystals of KH_2PO_4 and which, when injected into cats produced a marked fall in blood pressure, often with death of the animal. In a typical experiment they employed a solution containing 0.224 gm of KH_2PO_4 dissolved in 1 cc water (a 22 per cent solution) and obtained with a dose of 0.25 cc a marked fall in pressure. They also found that a solution of KCl in the same concentration produced a marked reduction in pressure when the same dosage was employed.

It seemed to us at the beginning fair to assume that if potassium ions are responsible for the fall in blood pressure observed after intravenous injection of liver extract then an aqueous solution of KH_2PO_4 or KCl containing these substances in the same concentration as they occur in the liver extract should have an equally strong depressor action.

We first analyzed a sample of liver extract with which we had been working (Heparphone of Eli Lilly & Company) and found that it had a potassium content equalling 0.9 per cent KH_2PO_4 . This liver extract was tested against 0.9 per cent aqueous solutions of KH_2PO_4 . As shown in Fig 1, the liver extract, in doses of 0.1 cc, 0.2 cc, 0.5 cc and 1 cc showed, on intravenous injection marked depressor effects, while a 1 per cent aqueous solution of KH_2PO_4 in the same dosage, produced no lowering of blood pressure. The 1 per cent aqueous solutions of KH_2PO_4 produced no depressor effect until a dosage of 2 cc was reached, the fall here being slight and corresponding in fall to that produced by 0.1 cc of liver extract. The liver extract then had a depressor effect equal to at least twenty times that produced by an aqueous solution of KH_2PO_4 whose content in potassium ions was equal to that of the liver extract (Figs 1 and 2).

In another experiment, the total solid content of the liver extract was determined and found to be 2.494 gm per 100 cc. An aqueous solution of KH_2PO_4 was then prepared containing 2.494 mg per 100 cc and its effect

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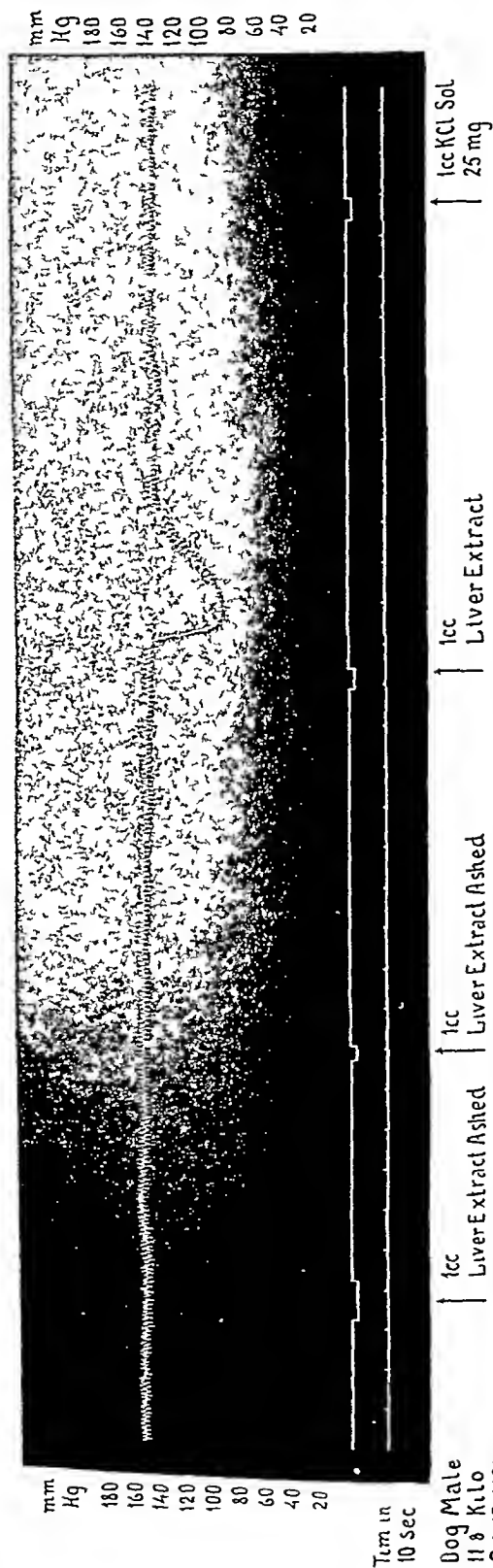


Fig 3 —Effect of ashed liver extract on blood pressure Intravenous injections KCl solution 0.9 per cent

Dog Male
11.8 Kilo
Oct 15 1923

upon the blood pressure noted. It was found when compared with liver extract, to have an extremely feeble depressor effect. This experiment shows that if the entire solid content of the liver extract were KH_2PO_4 , there is not enough present to account for its depressor effect.

We also ashed the solids of the liver extract and then redissolved the ash in distilled water making the solution up to its original volume. These solutions had no effect upon the blood pressure which would not have been the case if its activity were due to potassium ions. (Fig. 3)

In most of our experiments dogs were employed but the same results were obtained in the cat although the cat appeared to be somewhat more sensitive to the depressor action of potassium ions.

We can confirm the observations of Goerner and Haley that 20 per cent solution of KH_2PO_4 and KCl have a marked depressor effect upon the blood pressure of dogs and cats and may produce the death of an animal in from one to two minutes. The toxic effect of potassium ions upon the contraction of the heart is a well known physiologic effect and in our experiments the lowering of the blood pressure produced by 20 per cent potassium solutions was accompanied by a marked enfeeblement of the heart's action and, where the blood pressure fell to zero the heart promptly ceased beating.

CONCLUSIONS

1 The active depressor substance in the liver extract with which we have worked is not KH_2PO_4 since the liver extract in the doses employed does not contain enough potassium ions to produce the depressor effect which is obtained.

2 The fall in blood pressure obtained with 20 per cent solutions of KH_2PO_4 is due to what physiologists have long described as 'potassium inhibition' of the heart and has no close analogy with the fall produced by our liver extract. Such solutions of KH_2PO_4 contain at least twenty times as much potassium as the solutions of liver extract with which we have worked.

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CONCERNING CERTAIN FACTORS WHICH MAY INFLUENCE THE SUGAR CONTENT OF THE BLOOD AND URINE*

By E. M. WATSON, M.D. M.R.C.P. (Edin.) LONDON CANADA

OF THE several factors operating to cause variations in the blood-sugar concentration and in the output of sugar in the urine, the food intake is of prime practical importance. So important is the food factor that one is prone, perhaps to neglect the significance of those more obscure influences such as minute changes in the intra- or extracellular acid-base equilibrium, which are known to have an important bearing upon the processes of internal or tissue respiration and in directing the activities of the tissue enzymes.

While the fluctuations which occur in the blood and urine sugar are variable it is generally conceded that a temporary elevation of the blood-sugar concentration accompanied by a transient glycosuria, follows meals. It would appear, however that the extent of the increment of the blood sugar and the increase in the output of sugar is not the same following all meals of the day, even when the meals contain an equal amount of carbohydrate. For example Page¹ observing the hourly sugar excretion of individuals on a standard diet found that, in general the greatest increase seemed to follow breakfast. He noted also that the amount of sugar excreted was not entirely dependent upon the quality or quantity of the food ingested. Likewise, the daily blood-sugar curves obtained by Miller, Jonas and Teller,² from non-diabetic and from diabetic subjects showed, in a considerable proportion of cases the highest point to occur after the first meal of the day.

Those who are familiar with the practical problems related to the management of patients with diabetes mellitus know that from the laboratory standpoint at least the severe diabetic is frequently at his worst in the morning. The morning glycosuria is oftentimes difficult to control, even when the carbohydrate content of the breakfast is less than that of the other meals and the morning dose of insulin is greater than the amounts given at other times of the day.

From the foregoing remarks it would seem that there exists in many instances an inherent tendency toward hyperglycemia and glycosuria during the early part of the day which in the case of the individual with normal carbohydrate metabolism does not proceed to actual hyperglycemia and glycosuria but in the case of the individual with an unstable carbohydrate metabolism the tendency is exaggerated and the findings then fall within the range of the abnormal.

The explanation usually advanced for the more marked hyperglycemia and glycosuria of diabetics following breakfast is concerned with the sup-

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posed inactivity of the glycogenic function of the tissues during the night and its delayed reactivation in the morning. To quote the words of Gray,³ 'The fasting pancreas or liver is not ready for glycogen formation'. There is as it were, a latent period during which the absorbed glucose accumulates in the peripheral blood stream but once glycogen formation becomes started glycogenesis proceeds more or less effectively. This is analogous to what occurs in normal persons following the repeated administration of glucose. The ingestion of glucose appears to stimulate in some way the mechanism of carbohydrate disposal so that repeated ingestion of the same amount causes less marked hyperglycemia. This lag in glycogen formation by the tissues is of undoubted importance but it is not yet decided whether it is the only factor deserving consideration when searching for an explanation for the prominence of the morning hyperglycemia and glycosuria of diabetes.

Hatlehol⁴ in the course of an extensive investigation of the changes which occur in the sugar of the blood observed that the blood sugar concentration of fasting diabetics fell from morning to evening with the lowest level during the night, and that it rose again during the early hours of the following day. This increase he termed the 'paradoxical rise of the blood sugar concentration'. The phenomenon seemed to occur especially in the more severe cases of diabetes but it was observed in the milder ones as well and reappeared in one fasting period after another. The paradoxical rise was not demonstrated in healthy subjects. Hatlehol came up with no nearer explanation of the problem than to suggest that it is probably related in some way to an influence on metabolism closely connected with sleep or with the waking state. He recommended that periodicity and transition from one state to another should be taken into account when judging the effects of meals or other external factors on the blood sugar.

Search for a change in metabolism associated with the transition from the sleeping to the waking state which might have a possible bearing upon the question under consideration is not very enlightening. One is reminded however of the change in the acid base equilibrium described by Lethbridge⁵ as occurring at such a time. It appears that during sleep the respiratory center is inactive consequently CO₂ accumulates in the blood and a relative acidosis results. Upon awaking arising and resuming the duties of the day the respiratory center becomes reactivated CO₂ is washed out of the blood leaving the latter with a relative excess of base. Evidence of such an occurrence is the high CO₂ content of the alveolar air immediately upon awaking and its subsequent fall during the course of the morning. Accompanying this physiologic variation in the alveolar CO₂ is a change in the reaction of the urine resulting in the so called 'morning alkaline tide' which appears to be related to the alteration in respiratory activity which occurs at this time of the day rather than to any functional activity on the part of the digestive organs.⁷

Any change in the blood of sufficient consequence to cause detectable alterations in the urine must be capable presumably of exerting an influence upon the delicately balanced reactions which take place within the tissues. The morning is admittedly a period of instability, a time when physiologic readjustments must be occurring. It is to be expected therefore, that any

tendency to an unbalanced carbohydrate metabolism would be exaggerated at this time

Experimental evidence concerning an association between the respiratory function and sugar metabolism is not entirely lacking. Henderson and Underhill,⁸ as a result of their observations are of the opinion that "acapnia is a frequent concomitant of glycosuria or at least hyperglycemia both under clinical and experimental conditions." The experiments carried out by Imrie⁹ showed that voluntary hyperpnea in normal individuals caused an increase in the blood-sugar concentration accompanying a drop in the plasma bicarbonate. While experimental conditions are often remote from those ordinarily encountered clinically, the above findings point to a probable association between the acid-base equilibrium and certain phases of carbohydrate metabolism.

With such a probability in mind, it remains to be seen if the association in question can be applied in the elucidation of the practical problem of why the diabetic is worse in the morning. The observations which form the basis of this paper were carried out upon a series of nondiabetic hospital patients and upon an equal number of diabetic patients. None of the latter were of the "severe" type. Each patient received, during the two days of the experiment, meals of equal composition. Each meal consisted of protein 10 g, fats 25 g and carbohydrates 10 g. On the second day, breakfast was omitted. The patient emptied his bladder every two hours from 6 A.M. until 10 P.M. into bottles containing toluol. The urine secreted between 10 P.M. and 6 A.M. was collected as one sample. Samples of blood for blood-sugar estimations were withdrawn every two hours between 8 A.M. and 8 P.M. The blood-sugar estimations were carried out according to the Folin-Wu technique¹⁰ and the urine sugar was estimated by the method of Folin and Berglund.¹¹ The variations in the reaction of the urine may be considered to reflect, for the purposes of the present investigation, the changes in acid-base equilibrium, as referred to above. The method used for determining the reaction of the urine was that described by Leathes.⁶ The basis of this test rests in the fact that the urine reacts like a solution containing both mono- and di-basic phosphates. A measured volume of urine (10 c.c.) was diluted with two volumes of distilled water and two drops of phenolphthalein indicator and three drops of methyl orange indicator added. The mixture was then titrated with decinormal H_2SO_4 to the turning point of the methyl orange, then with decinormal $NaOH$ to the turning point of the phenolphthalein. The result of the first titration gives the equivalent of the alkaline phosphate while the second titration represents the total phosphate. The percentage of the total phosphate in the form of alkaline phosphate is designated the "alkalinity per cent." Calvert, Mayrs and Milroy¹² found this method to compare favorably with P_H determinations for purposes of studying alterations in the reaction of the urine.

Chart I is a diagrammatic representation, based upon the results of the above-mentioned observations, showing the changes which occur in the blood-sugar concentration, the hourly output of sugar in the urine and the urinary reaction, under the conditions of the investigation. The relationship between the three observed values is depicted. The curves of the diabetic group show

the same variations and follow the curves of the nondiabetic group but at a higher level. The events of the first day are not remarkable. There is shown the postprandial rise of the blood sugar and the accompanying increase in output of sugar in the urine. The morning alkaline tide occurs, the alkalinity per cent beginning to increase before any food has been taken. The fact that the chart shows the urine to be more alkaline in the diabetic group than in the nondiabetic group is regarded as coincidental and is not stressed. On the second day, the patients having received no breakfast the curves tend to conform in a general way to those of the first day with the exception of the blood sugar which does not rise so high in the morning as it does at the eve

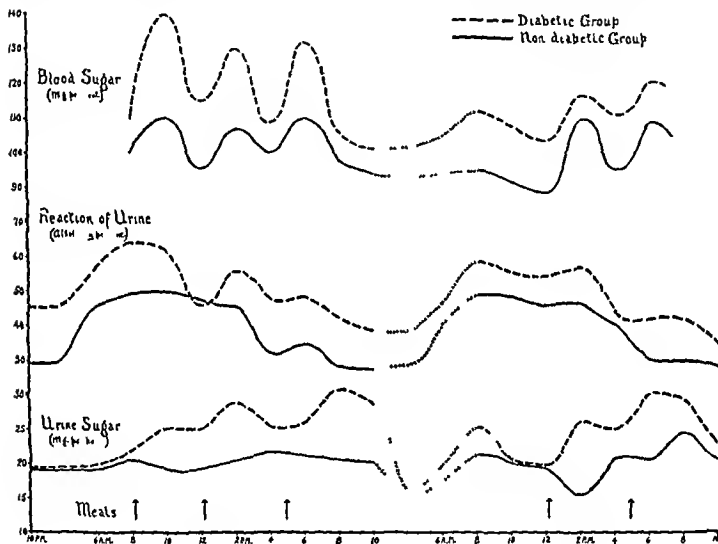


Chart I

responding hour on the first day but there is nevertheless, a rise more marked in the diabetic cases. There is also an increase in the sugar excretion in the morning compared with that during the night. It will be noted that the rise in the blood sugar and the increase in the urinary sugar correspond in point of time to the increase in the alkalinity of the urine. This relationship between the three variables fails at times of the day other than the morning.

One hesitates in the absence of more specific data, to emphasize the significance of an association between the reaction of the urine and the sugar content of the blood and urine. Benedict and Osterberg²³ noted that alkaline urines contained larger quantities of sugar than acid urines and that the transition to an acid reaction was almost invariably accompanied by a sharp fall in the rate of sugar elimination. In any case the problem deserves fur

ther consideration and it is possible, until the contrary is proved, that the alteration in the acid-base equilibrium which accompanies the change from the sleeping to the waking state may exert an influence upon the blood and urine sugar of diabetics if not upon normal individuals, thereby augmenting the effect produced by food

SUMMARY

1 The relationship between the blood-sugar concentration, the hourly output of sugar in the urine, and the reaction of the urine has been studied in a series of nondiabetic and in a series of diabetic patients on a standard diet

2 There appeared to be no constant relationship between the reaction of the urine and the sugar content of the blood and urine except perhaps during the morning

3 When breakfast was omitted, an increase in the output of sugar in the urine occurred, and in the case of diabetic individuals, there was an obvious rise in the blood sugar as well. These increments accompanied an increase in the alkalinity of the urine

4 Since it has been shown elsewhere that the morning alkaline tide of the urine appears to be related to an alteration in the acid-base balance associated with the change from the sleeping to the waking state, it is suggested that the same process of readjustment may be a factor in causing an increase of the blood and urine sugar during the early part of the day

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STUDIES IN THE PHARMACOLOGY OF LOCAL ANESTHETICS*

II IRRITATION AS PRODUCED BY LOCAL ANESTHETICS ON RABBIT'S CORNEA

By HAROLD W. COLES, PH.D. AND C. L. ROSE, INDIANAPOLIS, IND.

COCAINE has long led the field of local anesthetic compounds, as far as the efficiency in topical application has been concerned, but because of its extreme toxicity many substitutes have been proposed and tried. Some of these have been successful. Others have failed because their properties did not include the necessary effect when applied topically to mucous membranes. The assaying of these many compounds has entailed an immense amount of experimentation upon absorptive membranes, involving chiefly the corner of the rabbit^{1, 3, 4}. The greater part of these tests has been concerned primarily with the duration of anesthesia and has overlooked the accompanying degree of irritation, a consideration that is of great importance in evaluating a local anesthetic substance. This may have been due in part to the difficulty that such an experiment always presents in the way of recording. The photographic plate offers possibilities that have not been utilized in this field.

A simple method has been devised for recording irritation as evinced by inflammation of ocular and palpebral conjunctiva and pitting and mucus formation on the cornea and sclera. A diagrammatic drawing is made representing the conjunctiva and cornea. This is filled in with markings and color of varying degrees of intensity, to indicate the range of irritation and filed as part of a permanent record of a given local anesthetic.

Irritation has been defined as an exaggerated response to stimuli, but it must be more closely limited to make that definition apply to the rabbit cornea method¹. Irritation may be, but does not necessarily have to be accompanied by pain, by engorgement of the capillaries (incipient inflammation) of the cornea and conjunctiva, or by a temporary but none the less severe corrosion of the superficial cell layers over the iris and pupil. In response to pain the eye will be tightly closed, the lids being pressed together and wrinkled. Capillary engorgement is easily recognized by an increase in the intensity of the coloring of the palpebral conjunctiva and by an added redness on the sclera. Unfortunately it will be found more often than not, that the anesthetic showing the longer duration will also show a greater corrosive action on the cornea. The latter results in a temporary softening of the superficial cell layers over the iris, producing pits and cracks. Shreds of mucus may appear also. These conditions are recorded and upon their degree of presence or absence depends the value of local anesthetic compounds as far as irritation is concerned.

As a rule an individual under the identical conditions of dose and metab

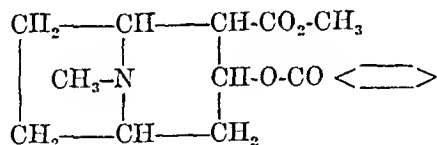
From the Eli Lilly Research Laboratories, Indianapolis, Ind.
Received for publication September 20, 1929

olism will always respond in such a way as to give an exact number of minutes' duration or a definite degree of irritation. But all individuals do not react in the same manner, and for the purposes of a physiologic experiment it is customary and best to employ a group of animals. For this particular type of work it was found that about 10 per cent of the rabbits used in the original groups gave durations that were as much as fifteen to thirty minutes longer or shorter than the other 90 per cent. The irregular 10 per cent were discarded. This left a group of animals which was constant in response under given conditions, and which might be said to be standardized by selection.

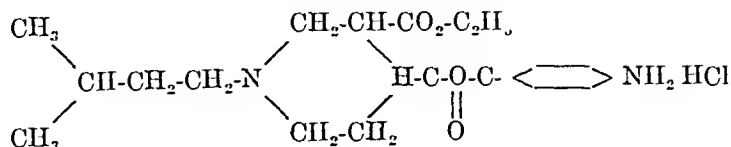
Following the method of Schmitz and Loevenhart, three rabbits in apparent good health were selected for each group, and isolated. The long hair and lashes were closely clipped from about their eyes, and pinching the lower lid to form a cup, 15 drops of a 2 per cent anesthetic solution were instilled into each eye. The solution was allowed to remain there for one minute and then was drained out. The eye was observed before instillation and a record made of its appearance in the normal state, then, after the local anesthetic had attained its maximum effect, the eye was again examined by the aid of an ophthalmoscope and a record made of its changed condition. In order that any disturbance occurring on the cornea might be attributed to the local anesthetic alone, a glass rod with a dull rounded end was used to stimulate the cornea when determining duration.

Twelve preparations have been selected to show the variations obtained by the instillation of different substances into the conjunctival sac of the normal rabbit eye. Plate I represents the degrees of irritation produced on the rabbit cornea and conjunctiva by

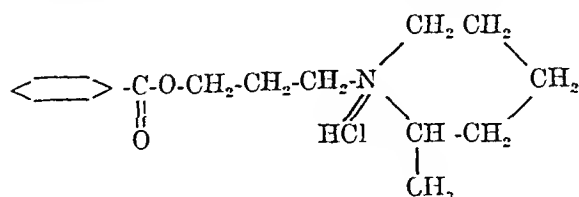
Cocaine,



- (5) #0 1-isoamyl-3-carbethoxy-4-piperidyl p-amino benzoate HCl,



- (6) #33G Gamma-2-(methyl piperidino)-propyl benzoate HCl,



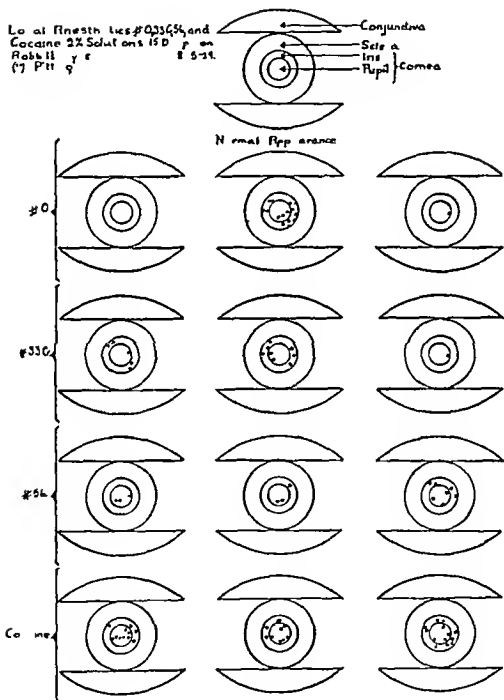


Plate I

(7) and ± 56 Gamma-(3-methyl piperidino)-alpha methyl propyl benzoate HCl,

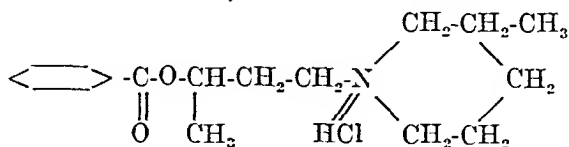
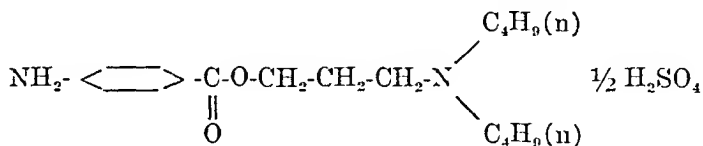
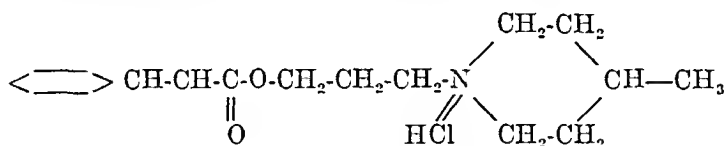


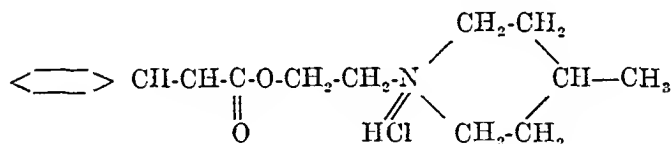
Plate II is a continuation of Plate I and represents the mutation caused by butyn,



* ± 93 4-methyl piperidino propyl cinnamate HCl,



* ± 92 4-methyl piperidino ethyl cinnamate HCl,



(8) and ± 58 1-phenyl ethyl-4-piperidyl p amino benzoate HCl,

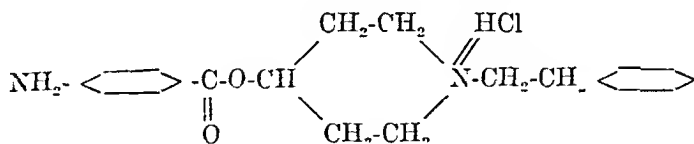
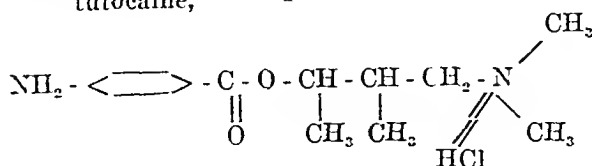


Plate III shows the results obtained from the use of distilled water normal physiologic saline, *Corvdales tuberosa* "B," and tutocaine,



From the accompanying plates it may be seen that each of the compounds tested has certain characteristics which produce the reactions seen in the eyes to which it has been applied

*The paper describing the chemistry of these compounds which were obtained from Professor S. M. McElvain of the University of Wisconsin for the purpose of physiologic testing is in print and will be published by him in the *Journal of the American Chemical Society*

Local anesthetics Butyl, #95
 95 and 98 2% Solutions 15
 Drops Rabbits eyes - 8-10-29

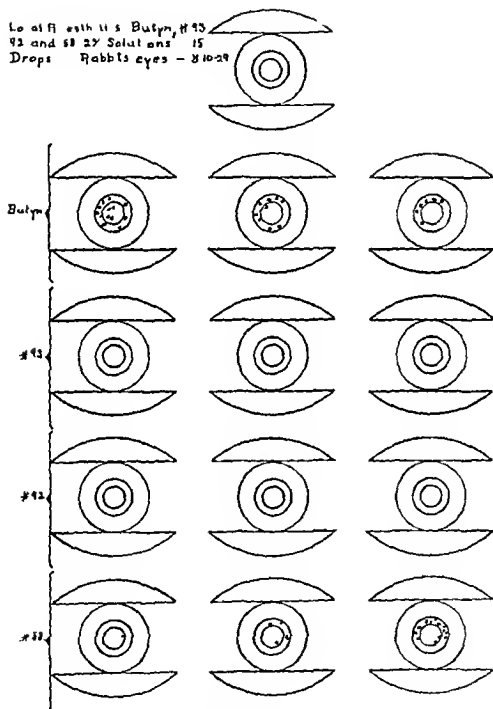


Plate II

By grouping the substances whose characteristics are most alike, they may be evaluated as follows

Group I—Very slight engorgement and no pitting

Distilled water

Normal physiologic saline

Group II—Slight engorgement and slight pitting

Tutocaine

#33

#56

Group III—Slight engorgement and severe pitting

Cocaine*

#0

Group IV—Severe engorgement and severe pitting

Butyn

#58

Group V—Very severe engorgement and little or no pitting

#93

#92

Corydalis tuberosa "B"

Extent of irritation, as defined in this paper, is not correlated, apparently, with the duration of anesthesia. *Corydalis tuberosa* "B," #93 and #92 show severe engorgement but are effective for only sixteen minutes or less. On the other hand, all those compounds producing more or less severe pitting are responsible for considerable duration. This is shown in the accompanying table.

COMPARISON OF PITTING AND DURATION OF ANESTHESIA

INSTALLATION MATERIAL	PITTING	DURATION
Distilled water	negative	negative
Normal physiologic saline	"	"
#93	"	13 minutes
#92	"	16 "
<i>Corydalis tuberosa</i> "B"	"	15 "
Butyn	3 plus	30 "
#33	1 "	40 "
#56	1 "	40 "
Tutocaine	1 "	40 "
#0	1 "	60 "
#58	1 "	60 "
Cocaine	1 "	60 "

CONCLUSION

1 Irritation produced by local anesthetics on the rabbit's cornea is given a limited definition in order that the term may apply specifically to the particular conditions here encountered.

2 A method for the permanent recording of irritation phenomena on the rabbit's cornea is described.

3 Irritation, in extent, is not paralleled by duration of anesthesia, although pitting is accompanied (with the materials studied), by a prolonged

*Cocaine possessing the property of being astringent to mucous membranes shows less engorgement than might otherwise be expected.

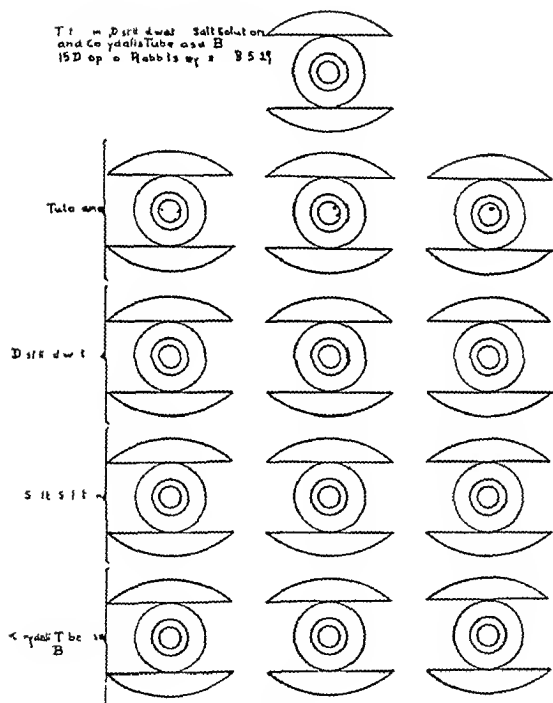


Plate III

anesthesia This may, however, show reversals, such as mild pitting and long anesthesia, as against very severe pitting with anesthesia of shorter duration

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LABORATORY METHODS

THE ESTIMATION OF HEMOGLOBIN A NEW HEMOGLOBINOMETER*

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THERE is probably no single laboratory procedure of greater importance than the estimation of hemoglobin and on the other hand probably none less satisfactory. The remedy for this condition has been eagerly sought, as is evidenced by the flood of articles which has swept the literature since the introduction of the first clinical hemoglobinometer by Gowers. However a satisfactory method seems never to have been developed. Those procedures giving results accurate to 1 per cent to 2 per cent require quantities of blood available only by vena puncture. On the other hand all those methods for which sufficient blood may be procured from a finger pricked, show errors of 5 per cent even when the most scrupulous care is exercised and constant vigilance is maintained regarding the standard solutions.

HISTORICAL

Ten different principles have been employed for the estimation of the hemoglobin content of blood. These may be enumerated as follows:

- 1 Direct color comparison method
- 2 Acid hematin method
- 3 Carbon monoxide method
- 4 Iron content method
- 5 Oxygen capacity method
- 6 Specific gravity method
- 7 Spectroscopic method
- 8 Spectrophotometric method
- 9 Refractometric method
- 10 Extinometric method

Since the literature is peculiarly lacking in any review of the large number of articles which have been published on this subject an analysis of the work is included here.

Direct Color Comparison Method—This principle was first introduced by Gowers (1878) who diluted a measured volume of blood until its color quality matched that of a standard picric acid solution. The dilution necessary gave a measure of the hemoglobin content of the blood. Von Fleischel later introduced a wedge shaped standard which in turn was modified to the form of the modern Fleischel Meischer instrument in which blood diluted to a given volume is compared in color to a standard wedge. This instrument is so calibrated that the hemoglobin content may be read directly from the wedge in grams of hemoglobin per 100 cc of blood.

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Burker (1924, 1925) adapted this principle to the Dubosque colorimeter so that the color of diluted blood might be compared to that of a standard. The standard used consists of a sealed constant-depth chamber which is introduced into the colorimeter below the prism.

In 1900 Tallqvist devised the simple procedure of blotting up a drop of blood on a piece of filter paper and comparing the color with a series of standard lithographed shades. The color values descend in 10 per cent gradations from a maximum 100 per cent which is indicated as the color of normal blood containing 5,000,000 red blood cells per cmm. This was later modified so that the color scale formed the circumference of a disc which might be enclosed in a watch-like case and so turned that any desired color value appeared at a small window (Roderick, 1926).

The Dare instrument (Dare, 1900) enjoys the most widespread use of any in this group. A drop of undiluted blood is drawn into the capillary space between two small plates of glass. One of these is milk glass, the other transparent. The plates are slipped into a groove in the instrument and the color of the blood compared through an eyepiece with that of a standard disc which may be so rotated as to give any desired color value.

The objection that shades of red are difficult to match colorimetrically hangs over all the instruments in this group and has caused their general supersedence by others of greater accuracy. The Tallqvist is probably the least reliable of the series, being basically little better than a crude estimation. The Dare instrument, however, seems to have gained considerable popularity in spite of its cost and the fact that it has many times been shown to have an accuracy little greater than the Tallqvist (Lindsay, et al, 1926, Mills, 1925, Osgood, 1926, Senty, 1923). In fact the general error of the instruments constructed on this principle has been shown by different observers to range from 10 per cent to 40 per cent. Osgood (1926), Mills (1925), Lindsay (1926) and others feel that the Dare hemoglobinometer gives readings which are accurate to only 20 per cent to 29 per cent. A number of other observers (Christensen, 1924, Lillhendahl-Petersen, 1914, Lindell, 1899, Luecy, 1923, Osgood, 1926, Senty, 1923) have found the Tallqvist method susceptible to errors ranging from 2 per cent to 40 per cent. In addition, Luecy has pointed out that the pierocarmine standard employed by Gowers fades on standing and that any slight acidity of the water used as a diluent causes a change in color quality.

Acid Hematin Method—Ever since the introduction of this method by Sahlb it has been the target for the majority of criticisms and modifications in the literature. Although this is a fair indication of the dissatisfaction it has afforded, it also marks this process as the most adaptable so that it has continued to find favor with clinicians in general.

The hemoglobin in the blood is converted to the brown acid hematin by the addition of 0.1 N hydrochloric acid and the color produced compared to a standard. The Lillhendahl-Petersen method (1914) utilizes as a standard a series of strips of paper of varying shades of brown. The blood is blotted up on a bit of filter paper which has previously been impregnated with a hydrochloric acid solution and its color value determined by comparison with the standard shades. Obviously, this, too is a crude estimation although some

workers have reported it to be more accurate than the Tallqvist (Christensen, 1924)

The acid hematin principle has also been adapted for use with the ordinary types of laboratory colorimeter. In such a case the blood is diluted after the production of the acid hematin and compared in color to a standard solution. This is usually prepared from a quantity of normal blood containing 5,000,000 red blood cells per cmm. Plesech (1910), Antenrich and Konigsberger (1910), and Haden (1922) have recommended the prism type of colorimeter (Hellge) as less expensive, although the regular Dubosque plunger type instrument is usually employed since it forms a necessary part of every laboratory equipment (Cohen and Smith, 1919).

The original apparatus of this type was devised by Sahli (1895). It consists of a small graduated tube in which the blood and acid are mixed. When the brown color of the acid hematin appears the solution is diluted by the addition of water until its color matches that of a similar standard solution in a sealed glass tube. The level of the liquid in the graduated tube gives a measure of the hemoglobin content of the blood.

Haessler and Newcomer (1916) and later Trimble (1922) modified this method so that the blood is diluted to a definite volume and its color then matched with one of a series of 10 standard tubes representing intervals of 10 per cent of the normal.

There are several disadvantages and sources of error which creep into this method. Chief among these is the fact that the color of the standard solutions fades rapidly, even though kept in hermetically sealed tubes. This has been definitely proved by a number of workers (Lindsay, et al, 1926, Terrill, 1922, Felton 1923) and although the rate of fading is quite constant it may average a 50 per cent loss of color in eighteen months. Many substitutes have been suggested for the standard acid hematin solution. Terrill (1922) uses a concentrated solution of acid hematin prepared by evaporating ordinary preparations preserving with glycerol and sealing in ampules. This is diluted for standards as required and according to this author loses 8 per cent of its color value in ten months. The same investigator has prepared a dried powder which may be weighed out accurately and dissolved to form a standard acid hematin solution. The stability of such a powder is somewhat greater than that of concentrated solutions kept in ampules. Several authors have advocated more simple substitutes giving the brown color of acid hematin. Felton (1923) suggests the alkaline oxidation product of pyrogallie acid, Jacobson (1919) prefers rufigallie acid solutions (hexa oxanthrachinon). A German investigator (Lipp Weingarten, 1918) advocates a solution of henna while a standard prepared from ferric and chromium sulphates has also been suggested (Haskins 1923, Osgood and Haskins, 1923). Standards of colored glass rods have been produced for the Sahli instrument (Leitz and Hellge, 1924) but the color match is difficult. Newcomer (1919-1923) has introduced as a standard a plate of brown semaphore glass into one side of a colorimeter. Osgood and Haskins (1923) and Robscheit (1920) consider the color in this modification too pale to allow of accurate determinations and it has been found that variations in the temperature of

solution cause perceptible errors. However, there are others who have welcomed this innovation.

The second great disadvantage in this method is the delay in the formation of the maximum color of the acid hematin when the blood and acid are mixed. It has been shown that the rate of formation of the color in an acid hematin solution follows a rectangular parabolic curve of the form $x = -ay^2$ and that the percentage error in the final reading at any moment is equal to $40/m$ where m denotes the number of minutes between mixing the blood and acid and taking the reading. Thus it may be seen that in order to produce a reading with an intrinsic accuracy to 1 per cent, forty minutes must be allowed to elapse before the reading is taken. This work has been corroborated by Meulengraecht (1916, 1921) and Komiya and Katakura (1922). Using this formula Newcomer (1923) has devised a table by which the reading at any time may be corrected to a maximum value.

Several workers have suggested heating the mixture in the water-bath to hasten the color formation. Berman (1919) advocates boiling for one minute. Osgood and Haskins (1923) advise a temperature of 55° to 60° C for seven minutes. Komiya and Katakura (1922) have shown that the maximum color formation occurs in five minutes if the solution is heated at 60° C, in ten minutes if at 50° C, and in fifteen minutes if 30° C is maintained. They feel, however, and are supported by Terrell (1922) that a cloudiness is produced by temperatures over 30° C, so that the color match is thus rendered more difficult. These same authors suggest that the reading be taken in one minute and the instrument so standardized as to give the correct values at this time. They have shown that the color formation in such a process runs parallel to the true hemoglobin value.

Several minor difficulties are also experienced. Differences of 1 mm in the bore of Sahli tubes have been shown to introduce an error of 25 per cent to 33 per cent unless the instrument is individually standardized (Kuttner, 1916). Staubli (1911) has shown that great care must be exercised in adding the correct amount of acid since the final reading is increased by only a slight excess of the acid. Terrell (1922) feels that the standard solutions tend to become turbid on standing only two months. Berezeller (1918) has stated that variations in the lipid content of the blood will alter the readings appreciably. And finally errors in pipettes, etc., obviously affect the results, but such errors are inexcusable in any well-regulated laboratory. Several slight modifications intended to facilitate estimations have also been described (Thro, 1925; Thisted, 1925; Kuttner, 1915).

The acid hematin method has been demonstrated under some conditions to give results showing errors which vary among the different observers from 5 per cent to 40 per cent (Berman, 1919; Christensen, 1924; Haden, 1926; Lindsay, et al, 1926; Muller, 1925; Osgood, 1926; Robseht, 1920; Lebermann, 1925). Notwithstanding, it is generally agreed that when carefully undertaken with instruments which have been scrupulously standardized and whose standards have been checked within a month, results are obtainable with errors within 5 per cent to 7 per cent.

The Carbon Monoxide Method—In 1892 Hoppe Seyler described a method for the determination of the hemoglobin content of the blood by saturating a known dilution of the blood with carbon monoxide. This converts the hemoglobin into carbon monoxide hemoglobin whose color is compared to a known standard. It proved too complicated for general clinical use until Haldane (1900) adapted it for use with the Gower colorimeter. Later Palmer (1917, 1918) modified the method for laboratory analysis to the Dubosque type of colorimeter. Hammerer and Schanlin (1924) suggested a series of standard tubes in a comparison rack such as is used for P_{H_2} determinations. In each case the carbon monoxide is supplied by bubbling coal gas through the mixture.

This method has found no great favor as a clinical process. Coal gas is not always available and although Miller (1923) has shown that acetylene may be used instead the method is somewhat cumbersome. Robscheit (1920) and Appleton (1918) state that the standard solutions deteriorate rapidly and must be renewed every month. Lucey (1923) has shown that the water for dilution must be slightly alkaline to litmus to prevent flocculation. If ordinary coal gas is used as a source of carbon monoxide a N/200 solution of potassium hydroxide is employed instead to neutralize the acidity caused by solution of the gas. On the whole this method gives results accurate to 5 per cent if the standards are fresh and the determinations carefully made.

Iron Content Method—Jacquet (1894), Hufner (1879, 1894), and Butterfield (1909) have shown that the iron content of hemoglobin is 0.334 per cent of its total weight. Since then Wong (1923), Berman (1919), and Fowweather (1926) have devised colorimetric methods for the quantitative estimation of iron in blood. These methods are too time consuming and require too great an amount of blood for routine clinical work but their accuracy is rather high (1 per cent) and they have thus found favor as a means of standardizing less accurate clinical instruments.

Oxygen Capacity Method—In this method the oxygen capacity of the blood is determined as a measure of its hemoglobin content since it has been shown that 1 gm. of hemoglobin combines with 1.34 cc. of oxygen (Hufner 1894). To Haldane and Smith (1899) goes the credit for the first reliable method of measuring the oxygen capacity but Van Slyke (1917, 1918, 1921, 1924) later modified the process so that it has become available for ordinary laboratory work.

Although no information has been given by Van Slyke as to the accuracy of his first instrument, reference is made to the work of Lundsgaard (1918) who shows in four series of duplicate determinations, intrinsic errors of 0.14 per cent, 3.0 per cent, 1.4 per cent and 0.7 per cent respectively. Later Van Slyke and Stadie (1921) published the results of duplicate analyses on a later modification of the original instrument. These show a maximum deviation of 2.1 per cent. Since then, however, an entirely new instrument has been devised which although more complex, shows a maximum variation in analysis results of 0.48 per cent (Van Slyke and Mill 1924). Its accuracy seems to have gained this process a place in modern methods of blood analysis, notwithstanding the fact that considerable experience and time are essential for trustworthy analyses.

Specific Gravity Method—A method for estimating the hemoglobin content of blood has been described by Lindell (1899) who accredits the process to Hammerschlag. This technique has been devised on the theory that the hemoglobin content and the specific gravity of blood have a definite relation. Since this assumption has been proved to be incorrect by the data of Meyer and Butterfield (1914) and Newham (1924) the method will not be considered further.

Spectroscopic Method—This principle is one of the oldest employed for the determination of the hemoglobin content of blood. Prever (1866), Quincke (1872), and Rajewsky (1876) have compared the spectrum of diluted blood with that of a known solution of hemoglobin. Sahlb (1895) has described the Hensch hematoscope in which a solution of blood in a wedge-shaped container is viewed through a spectroscope, the depth of the solution being adjusted by movement of the wedge until the two bands of oxyhemoglobin in the yellow portion of the spectrum are equal in width. Finally, Henri and Wurmser (1912) have devised a method in which the blood is diluted until the absorption band in the green portion of the spectrum disappears. Each of these methods, however, seems to have been short-lived, particularly since the large type of spectroscope employed rendered the apparatus bulky and expensive.

Spectrophotometric, Refractometric and Extinctometric Methods—These last three principles may be quickly dismissed from this discussion since they are seldom made use of in clinical determinations. The spectrophotometer compares the light value of the spectrum of an unknown hemoglobin solution with that of a standard (Matteis and Grunbaum, 1903). The refractometer is occasionally used to standardize less accurate clinical instruments (Stoddard and Adair 1923, Giam, 1925, Howard, 1920). The refraction afforded a beam of light by an unknown solution of hemoglobin is compared to the refraction produced by a standard solution. The least adaptable of the three is the extinctometer (Wolviu, 1924). In this instrument the absorption of light by a solution of unknown hemoglobin content is measured by the intensity of the transmitted rays and compared to known standards. Each of these methods while obviously accurate, requires an operating time and a technical knowledge too great to allow of general use.

DESCRIPTION OF AUTHOR'S METHOD

A method has been devised by the author which makes use of the spectroscopic principle and which shows an accuracy comparing favorably with any existing technique. A discussion of its principles and procedures follows.

Theoretical—Oxyhemoglobin is one of the few compounds whose spectrum shows a marked variation in the number and width of the absorption bands with changes in the concentration of the solution. This was first observed by Hoppe-Seyler (1862-1864) and later by Stokes (1864). Rollet in 1880 undertook a quantitative study of these changes and has produced a series of curves plotting the variations in the absorption bands with concentration of the solution under analysis. Fig 1 is a reproduction of the curve for oxyhemoglobin for a fluid depth of 1 cm. To ascertain the amount of absorption for any given concentration up to 1 per cent, a horizontal line may

be drawn across the diagram at the level corresponding to the concentration. Where this line passes through the shaded part of the diagram, absorption takes place, and the width of the absorption bands is seen at once. The diagram clearly shows that as the concentration is increased three bands become apparent, two in the yellow portion of the spectrum between the D and E lines and one in the extreme blue. These become wider until at a concentration between 0.6 per cent and 0.7 per cent the two bands in the yellow portion fuse and for a greater concentration between 0.8 per cent and 0.9 per cent, they are joined by a band in the blue.

Beer in 1852 showed that there exists for any solution a simple relation between the absorption, the concentration and the depth of the solution under analysis. This may be expressed by the equation

$$-\log T_{\lambda} = k_{\lambda} c d \quad (\text{Equation 1})$$

where T is the light transmittance for the particular wave length c is the concentration of the solution, d represents the thickness of the fluid stratum and k_{λ} is a constant for the working wave length.

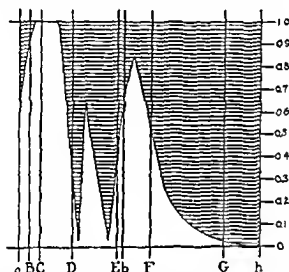


Fig 1—Diagram to show the variations in the absorption spectrum of oxyhemoglobin with varying concentrations of the solution (After Rollet). The numbers to the right give the strength of the oxyhemoglobin solution in percentages; the letters give the positions of the Fraunhofer lines. To ascertain the amount of absorption for any given concentration up to 1 per cent, a horizontal line may be drawn across the diagram at the level corresponding to the concentration. Where this line passes through the shaded part of the diagram absorption takes place and the width of the absorption bands is seen at once.

For the conditions of this experiment, in which the transmittance is a constant and attention is limited only to a single portion of the spectral field, the equation becomes

$$cd = A \quad (\text{Equation 2})$$

where A is an absorption constant.

Applying this to the foregoing, it is evident that absorption is proportional to the concentration as well as to the fluid depth so that the same changes in the absorption bands may be elicited by variations in the depth of the fluid stratum as are caused by changes in the concentration.

It was shown by Reid (1905) that oxyhemoglobin forms a true solution and may thus be reasonably considered to follow this same law. Some time later Butterfield (1912) was able to prove that blood itself in dilutions from 0 to 200 acts as a true solution and follows this relation between absorption, fluid depth, and concentration.

The idea immediately presents itself that the oxyhemoglobin content of blood may be determined by means of this equation. If blood be diluted to a known volume and the depth of the fluid stratum varied until the spectrum caused by it assumes some predetermined picture, the concentration of the oxyhemoglobin in the solution will be inversely proportional to the known depth. Or

$$c = A \frac{D}{d} \text{ (Equation 3)}$$

where c = the concentration of oxyhemoglobin in the blood (gm per 100 c c)

A = the absorption constant (determined experimentally)

D = the dilution (c c)

d = the depth of the fluid stratum (cm)

This principle has been employed by the author to determine the hemoglobin content of blood, since the oxyhemoglobin and hemoglobin contents are

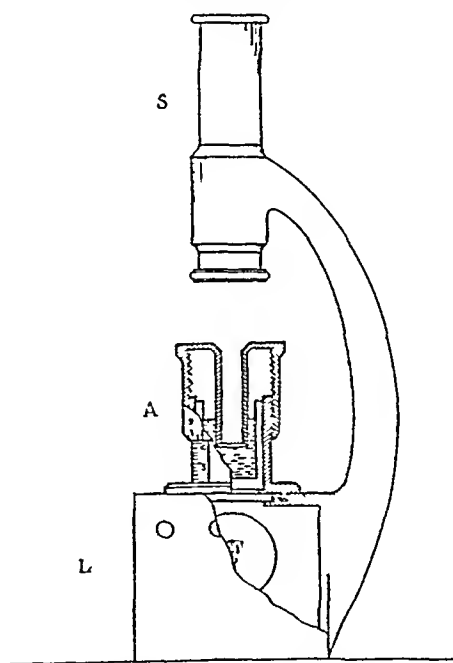


Fig 2—Sketch of the authors instrument. A direct vision hand spectroscope S is so mounted that the spectrum of the fluid in the absorption chamber A may be viewed through it. Illumination is furnished by a small 20 watt microscope lamp enclosed in a case at L . The completed instrument is furnished by E. Leitz, Inc. 60 East Tenth Street New York City.

obviously interlinked. The fluid stratum of blood, diluted to a known volume, is varied until the two absorption bands between the D and E lines are just separated. The hemoglobin content is then estimated from the foregoing equation (Equation 3), the constant A having been determined experimentally.

Description of the Instrument—Fig 2 is a sketch showing the assembly of the instrument devised by me for estimating the hemoglobin content of blood. A small direct vision spectroscope, S , is so mounted that the spectrum of the fluid in the absorption chamber A , may be viewed through it. The variation in the fluid depth is produced by screwing down or up the cap of

the plunger. Readings are made from the micrometer scale on the side of the instrument. It has been found essential to have a fixed source of light, *L*, since variations in the illumination such as would be incident with ordinary daylight, cause considerable errors in the readings.

The Method—In this procedure 0.1 cc* of blood is added to 3 cc of water and the two mixed by stirring. Laking is almost instantaneous in this dilution. This fluid is placed into the absorption chamber of the instrument and its spectrum viewed through the spectroscope. The plunger of the ab-

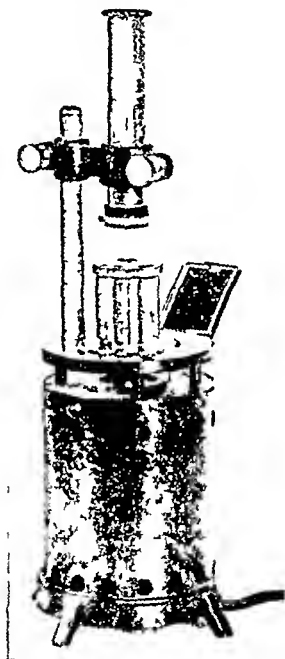


Fig. 3—Photograph of hemoglobinometer used by the author

sorption chamber is screwed down until a fine line of yellow light just appears between the two absorption bands. At this point the reading of the fluid depth is taken and from it the hemoglobin content calculated by means of the following relation derived from Equation 3

$$c = 31 \frac{A}{d} \quad (\text{Equation 4})$$

Experimental—Obviously this necessitates the determination of the constant *A* of the foregoing equation. Since variations in the light have been

*Since the completion of this article the amount of blood required has been reduced from 0.1 cc to 0.05 cc. by reducing the size of the absorption chamber.

shown to affect the hemoglobin readings, this constant should be determined for each individual instrument. This may be accomplished by the analysis of bloods or solutions of known hemoglobin content, with subsequent substitution of the results in Equation 4. It must be borne in mind, however, that in such a case the constant has an accuracy no greater than that of the method employed for the determination of the hemoglobin content of the standard blood.

The constant for the author's instrument having been determined, a group of experiments was undertaken to ascertain the accuracy of the process. Solutions of known hemoglobin value were prepared from weighed amounts of pure crystalline oxyhemoglobin and analyzed by this method. Table I shows how close is the agreement. Even on bloods of low hemoglobin content the readings check within 0.7 per cent which is an accuracy obtainable with few clinical hemoglobinometers. In fact methods not generally used clinically, which require bloods in amounts from 2 cc to 5 cc give results no better than this.

Experiments were then undertaken to ascertain the effect of jaundice upon the hemoglobin estimation by this method. It is well known that the results of any of the colorimetric methods are unreliable in the presence of bilirubin in the blood serum. Varying amounts of bile obtained through can-

TABLE I
ACCURACY OF ANALYSES

HEMOGLOBIN CONTENT GM PER 100 C C		DIFFERENCE
GRAVIMETRIC	SPECTROSCOPIC	PER CENT
20.032	20.09	0.24
15.024	14.95	0.49
10.016	10.06	0.44
7.512	7.46	0.70

nulation of the common duct of an anesthetized dog were added to measured quantities of blood. Equal amounts of physiologic saline were added to another series as controls and the hemoglobin contents of both groups analyzed. Table II gives the results. It is seen that bile present in amounts up to 20 per cent of the total volume of the blood has no effect upon the accuracy of this method. This is a distinct advantage since heretofore accurate determinations on jaundiced patients have been possible only by using some method other than colorimetric, all of these requiring more blood than can be procured from a finger prick.

In addition, such an instrument is useful for the qualitative analysis of such substances as hemoglobin in the urine or elsewhere, for methemoglobin, carbon monoxide hemoglobin, etc. Methods for the quantitative analysis of these last two substances are being developed.

There are several criticisms which might arise from a consideration of this method. For, quite obviously, if the hemoglobin of the blood were not totally saturated with oxygen so that it had been entirely converted to oxyhemoglobin, considerable errors would be introduced. It has been found, however, that enough oxygen is absorbed by the blood during its exposure on the finger tip and from the water with which it is diluted to cause no appreci-

able error in the results. On the other hand, the oxygen dissolved in 3 c c of water is more than sufficient to oxidize the reduced hemoglobin of the blood of even cyanotic patients.

TABLE II
EFFECT OF JAUNDICED BLOOD ON HEMOGLOBIN ANALYSES

PER CENT BILE IN BLOOD	HEMOGLOBIN ANALYSIS RESULTS ON PER 100 C C		PER CENT DIFFERENCE
	JAUNDICED BLOOD	CONTROL BLOOD	
4.7	13.39	13.50	0.90
9.10	12.96	13.00	0.31
20.00	11.21	11.11	0.54

Finally it would seem that any changes in the temperature of the blood solution would cause errors in the results of the analyses. However, the ordinary variations in room temperature have been found to introduce no perceptible change in the hemoglobin reading. From a theoretical standpoint the difference in fluid density resulting from a change of 10° C in the solution temperature would cause a variation in the final result of only 0.1 per cent which is well within the instrumental error.

CONCLUSIONS

This spectroscopic method for the determination of the hemoglobin content of blood has the following advantages:

- 1 It is simple, requiring no technical knowledge.
- 2 Its error is less than 0.7 per cent.
- 3 It is rapid, a complete analysis requiring approximately three minutes.
- 4 It requires no chemical solutions or colorimetric standards which are liable to fading and require replacement.
- 5 It requires only as much blood as can be drawn from the finger tip (0.1 c c).
- 6 Its accuracy is unaffected by the presence of bile pigments in the blood.
- 7 It may be used for all spectroscopic qualitative tests.

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A MICRO-MODIFICATION OF B GRUSKIN'S DETERMINATION OF UREA IN BLOOD*

BY HENRY TAUBER, PH D , AND BORIS KWARTIN, M D , BROOKLYN, N Y

A METHOD for the estimation of urea in blood has been worked out by B Gruskin,¹ using 1 c c of oxalated blood in his method. We modified this method by using 0.2 c c of blood which is drawn from the finger tip in the usual manner.

PRINCIPLE

Urea is decomposed to ammonium carbonate by means of urease, the proteins are precipitated, and the urea is determined colorimetrically by direct nesslerization.

REAGENTS

- 1 *Jack bean urease solution* (Fohn)
- 2 *A precipitation mixture* used for the preparation of the protein-free filtrate is made up in the following manner. Use 7 parts of distilled water, 1 part of a 10 per cent sodium tungstate solution, and 1 part of $\frac{2}{3}$ N sulphuric acid. Shake thoroughly.
- 3 *Nessler's reagent* (Koch-McMeekin)
- 4 *Stock ammonium sulphate solution*

Ammonium sulphate, C P	0.4716 gm
Concentrated hydrochloric acid	10 c c
Water, ad q s	1000.0 c c

Micro standard is made by taking 10 c c of this solution and diluting it to 100 c c. One hundred c c of the micro standard equals 1 milligram nitrogen.

The ammonium sulphate should be dried in hot air for one-half hour at 110° C.

METHOD OF PROCEDURE

Two-tenths c c of oxalated blood is placed in a specially constructed, ground glass stoppered, 15 c c graduate centrifuge tube, containing 1 drop of a 12 per cent potassium oxalate solution, and shaken. Add 0.2 c c of the jack bean urease solution and place in a water-bath of 45° to 50° C for ten to fifteen minutes. One and six-tenths c c of the precipitation mixture are added, the tube is stoppered and inverted several times. Let stand 3 minutes. Centrifuge for two or three minutes at high speed. Pipette off 1 c c of the clear, colorless supernatant fluid and place in a 5 c c graduate centrifuge tube. Add 2 c c of distilled water and then 0.6 c c of Nessler's reagent. Dilute to mark 5 and read in the colorimeter against the standard solution, which is set at 20. For standard, take 1.5 c c of the micro standard, 2 c c of distilled water, and 1.5 c c of Nessler's reagent in the order given †.

*From the Biochemical Laboratory, Beth Moses Hospital, Brooklyn, N. Y.

†If the urea is very high it is advisable to dilute the supernatant fluid.

CALCULATION

$\frac{\text{Standard Reading}}{\text{Reading}}$ times 15 equals mg urea per 100 cc of blood

COMMENT

The method is very simple and easily applied, the technical procedures are reduced to a minimum. The use of the precipitation mixture in place of the separate reagents is of great advantage. The calculation is the same as in the macromethod because the relative proportions remain the same. Numerous parallel determinations from oxalated venous blood and capillary blood obtained by finger puncture show the differences in the reading so minute as to be negligible.

COMPARATIVE VALUES OF MACRO AND MICROMETHODS

SPECIMEN NO	UREA MG PER 100 CC OF BLOOD		DIFFERENCE
	MACRO	MICRO	
1	15.2	15.2	0
2	14.2	14.5	0.3
3	15.6	16.1	0.5
4	13.95	13.25	0.70
5	15.15	14.5	0.65
6	13.5	15.2	1.7
7	50.0	54.0	4.0
8	39.1	40.2	1.1
9	26.8	27.6	1.2
10	8.0	9.3	0.7

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OPERATION OF APPARATUS

Tighten clamp *T* open clamp *U* open stopcock *F*, evacuate the system through pressure tube *O* until there is a drop in the mercury manometer to about 20 mm. While the evacuation is taking place, the cover, *X*, settles firmly on the jar, *Q*, tighten clamp *R* further and remold the putty on the seam between cover and jar. Shut off the vacuum at *O*. Watch the mercury manometer to detect any leak in the apparatus. Heat the brass tube, *H*, to a dull red heat by means of a Bunsen burner, *V* (better still a blast lamp). Open clamp *T*. Open the hydrogen tank and allow the gas to flow at a slow rate. Stop the flow of hydrogen when the mercury in the manometer returns to atmospheric pressure. After a minute or so open the hydrogen tank again and allow a few more cubic centimeters of hydrogen to flow into the jar. Shut off the hydrogen tank and tighten clamp *T*. After two or three minutes tighten clamp *U*. The jar, *Q*, and stand, *S*, may now be removed with the connecting tube, *N*, and the clamp, *U*, attached.

Instead of the connecting tube, *M*, a glass manifold, *W*, may be substituted and in this manner several jars may be rendered anaerobic at the same time.

The palladized asbestos in tube *H* can be periodically examined by forcing air through *O*. This expels the copper mesh with the palladized asbestos through *K*.

As a test for anaerobiosis in the jar, a tube containing 10 c.c. of 1 per cent glucose broth colored with 0.1 c.c. of 1 per cent aqueous methylene blue is placed in the jar. The liquid should become completely decolorized in a few hours in the incubator at 37° C.

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A SUGGESTION FOR COLLODION SACS*

By MILES J. BREUER, M.D., LINCOLN, NEBRASKA

THERE are certain clinical laboratory methods depending on the use of a collodion sac or capsule, which are not used as widely as they ought to be, because of the difficulty in making these sacs successfully. The research man who spends some months on a problem in which these sacs are required and during which time he needs large numbers of them, may develop the skill and



Fig. 1



Fig.

facility necessary to make them successfully. The man engaged in clinical practice, who needs a sac only occasionally, finds great difficulty in getting constant and dependable results in making collodion sacs.

I have talked with a number of men in charge of clinical laboratories and find that the reason that they are not interested in such procedures as the dialysis method for the P_H value of the blood or the Abderhalden reaction, is that they are unable to make the sacs. After finding my own efforts to make

these sacs most uncertain, I corresponded with a number of laboratory men, but was quite unable to get any real assistance or information on the subject. Those who are successful in making these sacs, consider it easy and cannot understand why some one else cannot make them, and yet they do not seem to be able to get their technique down on paper.

In casting about for some method for securing dependability of results in making these sacs, I hit upon the expedient of using a paper support for the collodion. This method, with a slight amount of practice, works uniformly, and gives a strong, rigid sac, much easier to handle than the unsupported kind.

There is a good deal of literature on the subject of making collodion sacs, but everything I have been able to find concerns itself with the *permeability* of the collodion membrane, and therefore with the composition of the collodion mixture entering into its making and with the methods of drying, hardening, etc. This paper does not concern itself in any way with the question of per-



Fig 3

meability, but merely with a support for any type or kind of collodion membrane that is necessary for any particular procedure. All the modifications of collodion mixture and all the variations of permeability are quite applicable in this method, for the tissue paper support is in no way concerned with the permeability of the sac. It merely acts as a coarse framework to support the collodion membrane.

The method is extremely simple and is far easier to carry out than to explain. Use a small test tube as a mold on which to form the wet tissue paper support. The tube in which commercial Loeffler's coagulated blood serum for diphtheria cultures is purchased makes a convenient size, being about 0.75 by 9 cm. A thin toilet paper makes a satisfactory paper.

In order to make the bottom of the paper capsule tight so that the collodion will not leak, it is necessary to lay three strips of the tissue paper, about 0.5 by 10 cm over the bottom of the tube, as shown in Fig 1. Wet the strips and they will adhere to the tube and remain in place. In the illustration only

two strips are shown for the sake of clearness, a third strip should be put on, and all of the glass surface covered

Then lay the tube on a piece of damp tissue paper, allowing an inch to project over at the bottom, as in Fig 2 Roll one turn about the tube, and then fold the projecting portion back on the tube, as in Fig 3 The rolling is then finished, and the tube with the paper on it, as in Fig 4, is allowed to dry After it is dry the glass is readily removed and we have a paper capsule, Fig 5



Fig 4



Fig 5

Now, follow the procedure usually used in making the collodion sac in a glass tube Fill the paper form with collodion for a few seconds empty, and drain upside down for one to three minutes If the mouth of the tube shows a tendency to become too hard and dry, dip into collodion for a centimeter of its length, while the upper portion is setting After it has set for not over three minutes, repeat Usually this is done three times, but of course here we are entering the domain of permeability, which has no business in this article After the collodion has set for the last time, the sac is placed in distilled water, where it is kept until needed

A MODIFIED VARNEY JAR FOR THE CULTIVATION OF ANAEROBES BY MEANS OF PHOSPHORUS*

BY S R DAMON, PH D, BALTIMORE, MD

BEGINNING with the work of Sellards,¹ stick phosphorus has been used by several workers as a means of reducing oxygen tension in closed containers in which anaerobes were to be cultivated. In an attempt to simplify the technic and employ apparatus easily obtained by any laboratory, Bushnell² introduced the use of a pressure cooker or fruit jars in place of Sellards' more elaborate container. As an improvement over this, Varney³ suggested the use of the ordinary museum jar five by twelve inches in size with a removable wire rack which would contain the tubes or Petri dishes to be incubated. In this laboratory this apparatus has been successfully employed in the cultivation of anaerobic spore-forming bacteria and spirochetes from the mouth.

The use of the Varney apparatus has not been unaccompanied by difficulties, however. In some instances the rubber gasket has not formed a perfect seal, and although the phosphorus ignited and was consumed, it was found that leakage occurred and we did not actually have anaerobic conditions. Our chief difficulty and expense, though, has been with breakage of the jars. In the hands of students this was not uncommon and led us to the design of a galvanized iron can that could be substituted for the museum jar. At the same time we incorporated features that enabled us to use it not only for anaerobiosis but for the cultivation of organisms favored in their growth by an increased CO₂ tension.

The apparatus is illustrated in Fig 1. At the left is shown the museum jar as described by Varney with its internal heavy wire rack supporting the inverted tin cylinder which protects the cultures from the deposit of phosphorus pentoxide. On top of this is an asbestos ring supporting an evaporating dish and surrounded by a wire gauze screen which protects the sides of the jar from spattering phosphorus during combustion. This figure is included to show the internal assembly which is not shown in our galvanized iron container shown in the center illustration. The essential features of this container are that it is made of No. 26 gauge galvanized iron with a tightly fitting lid of the same material which overlaps the cylinder about one and a quarter inches. Obviously a lid of this construction will not be air-tight and modeling clay is used over the joint to render it so. This is quickly applied and smoothed out after the lid is placed in position and the seal is generally formed before the phosphorus ignites.

Two features of the lid should be emphasized. In the first place it is made with a projecting flange at the top, as shown in the illustration. This

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affords a grip that is an aid in removal. In the second place it has two air tight stopcocks soldered into it. These are useful in connecting the apparatus to a source of CO_2 and a graduated cylinder, immersed in water, when it is to be used in the cultivation of organisms favored by an increased CO_2 content in their environment. Under such circumstances when the stopcocks *A* and *B* are opened and *C* closed, after submergence of the graduated cylinder in water, the entrance of CO_2 through *A* displaces air through *B* and the proportion desired may be obtained by measuring the amount collected in the cylinder.

For details of construction of the wire rack and tin cylinder reference should be made to Varney's paper, as to give the instructions here would be mere repetition. Suffice it to say then that when constructed to fit a galvan-

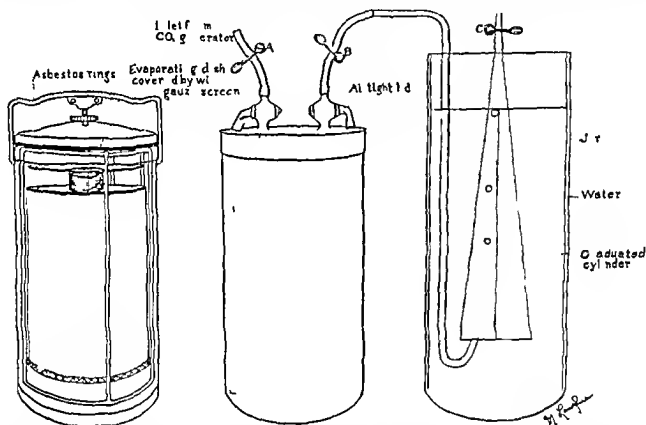


Fig 1—The left hand figure represents the complete assembly of the Varney jar. The center figure shows the details of construction of the galvanized iron container with suggested connections. The right hand figure indicates the method of collecting displaced air when an increased CO_2 atmosphere is desired in the jar containing the culture.

ized iron can six and one half by twelve inches we habitually place twelve to fifteen plates in each container.

In the use of the apparatus the usual precautions in handling phosphorus should be observed to prevent premature combustion and burns. It may also be said that we have found it advantageous to place the whole apparatus in the ice box for a short period before opening it after it has been in the incubator. This chilling allows more leeway in the process of opening it up before combustion of the residual phosphorus.

Briefly stated the steps in the assembly of this apparatus are

- 1 Place a little water in the galvanized iron jar
- 2 Load the Petri dishes or tubes on the wire rack
- 3 Place the tin cylinder over the cultures
- 4 Put the rack of cultures in the galvanized iron jar

- 5 Place the asbestos ring on top of the tin cylinder and put the evaporating dish in place
- 6 Put the piece of phosphorus in the evaporating dish and cover with the wire screen
- 7 Close the stopcocks in the jar lid and place it in position
- 8 Complete the air-tight seal with modeling clay
- 9 Place jar at desired incubation temperature

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THE APPLICATION OF THE PROCESS OF CIRCULATORY SOLUTION TO THE PARAFFIN INFILTRATION OF TISSUES*

BY WILLIAM F SHERIDAN, A B, WASHINGTON, D C

THE infiltration of tissues with paraffin can be facilitated by the application of the process of circulatory solution

The process is effected by suspending cleared pieces of tissue upon a porous diaphragm near the surface of melted paraffin. The portions of the clearing reagent coming into immediate contact with the melted paraffin are diluted and distributed, and descend, their places being supplied by fresh portions of melted paraffin. A circulation is created and dilution and distribution of the clearing reagent throughout the paraffin facilitated.

The clearing reagent being miscible in the melted paraffin is displaced and the interstices of the tissues filled with paraffin containing varying amounts of clearing reagent, depending in extent upon the proportion of paraffin used in effecting the infiltration. Complete or nearly complete replacement of the clearing reagent within the interstices of the tissues can better be effected by using large volumes of melted paraffin. This will result in greater dilution of the displaced clearing reagent, and its subsequent removal from the melted paraffin will be expedited by exposing a greater surface to the air.

The paraffin bath as generally practiced one, two, or three changes in shallow containers of very small capacity, with the tissues resting on the bottoms of the containers, obviously cannot result in complete replacement of the clearing reagent in the optimum period of time. Dilution of the displaced clearing reagent by its distribution throughout the melted paraffin is not accomplished. During part of the time considered necessary to complete the infiltration, the tissues are bathed in paraffin that contains an excessive amount of clearing reagent at the bottom of the containers and which is incapable of

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dilution and distribution throughout the too small volume of paraffin, due (particularly chloroform s.g. 1.490) to the difference in density.

The apparatus illustrated in this paper has, over a period of two years, given results that were very satisfactory. It is inexpensive, and correct in principle for the establishment of circulatory solution. The pan, measuring 24 cm long, 14 cm wide and 6.5 cm deep, is filled with 1000 gm of paraffin, which when melted brings the surface within 2 cm of the top of the pan. The

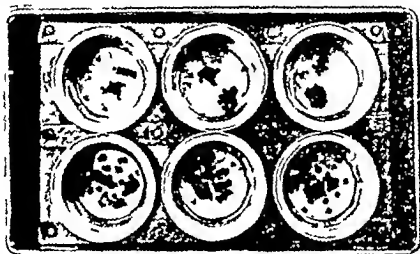


Fig. 1

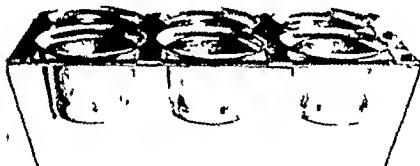


Fig. 2

rack is made of brass 12 cm wide by 0.1 cm thick. Two strips 34 cm, one strip 22 cm and four strips 12.5 cm long, are bent and riveted together so that the whole conforms to the inside dimensions of the pan, furnishing six spaces each measuring 6.3 cm by 4.5 cm. Six aluminum cups with shoulders, measuring 6.3 cm across the top, 4.4 cm across the bottom, and 4.4 cm deep with numerous small perforations on the bottoms and sides are suspended in the six spaces of the rack. The whole is then placed in an oven or other heating appliance that will maintain a temperature at or near the melting point of the paraffin.

WASSERMANN TEST WITH GLYCERINATED HUMAN SERUM*

BY E HENRY RUEDIGER, M D, HOLLYWOOD, CALIFORNIA

ALTHOUGH the different steps of the Wassermann test with glycerinated human serum (Ruediger's modification of the Wassermann test) have been described previously, a concise description of the entire method seems to be in order, because the others are scattered over a period of several years

Shortly after having begun to do the Wassermann test, I saw the importance of preserving the human serum to be sent to distant laboratories, to be used as control serums and in order to establish a unit of measure which may be used in the standardization of the Wassermann test

Upon trying a number of different preservatives, I¹ found glycerol to be quite satisfactory for the purpose Glycerol renders the serum moderately anticomplementary, which must be overcome by increasing the quantity of hemolytic amboceptor The titration of hemolytic amboceptor must be done in the presence of as much glycerol as is used in performing the test

Early in 1916, I² showed that glycerinated human serum keeps well for several months In other reports, I^{3, 4, 5} showed that glycerinated human serum holds its titer fairly well, provided it is kept at low temperature, preferably below zero Fahrenheit Dried⁶ human serum holds its titer much better

ANTIGEN

In my studies on antigens,^{7, 8, 9} I found alcoholic extracts of syphilitic human heart muscle and alcoholic extract of normal beef heart muscle most satisfactory A small quantity of cholesterol, 25 mg per 100 cc of extract, may be added to advantage The antigen is diluted slowly to give an opalescent solution, and is used in the optimum dilution In order to obtain uniform results, uniform methods must be used In diluting the antigen, I proceed as follows Put 0.1 cc of extract (antigen) into a 25 cc graduated cylinder,

TABLE I
ANTIGEN TITRATION

NEGATIVE CONTROL SERUM PRECISION METHOD							POSITIVE CONTROL SERUM PRECISION METHOD							
ANTIGEN DILUTION	READINGS						RESULTS	READINGS						RESULTS UNITS PER CC
	ANTIGEN TUBES			CONTROL TUBES				ANTIGEN TUBES			CONTROL TUBES			
	1	2	3	1'	2'	3'		1	2	3	1'	2'	3'	
1 20	0	0	+	0	0	±	Anti comp	0	±	+	0	0	±	10
1 30	0	0	±	0	0	±	OK	0	+	+	0	0	±	15
1 40	0	0	±	0	0	±	OK	0	+	+	0	0	±	15
1 60	0	0	±	0	0	±	OK	±	+	+	0	0	±	20
1 80	0	0	±	0	0	±	OK	±	+	+	0	0	±	20
1 120	0	0	±	0	0	±	OK	±	+	+	0	0	±	20
1 160	0	0	±	0	0	±	OK	0	+	+	0	0	±	15

Explanation 0 means complement is not fixed or hemolysis + means complement is fixed or no hemolysis ± at least 50 per cent fixation TR less than 50 per cent fixation

*From the Hollywood Clinical Laboratory

add 0.1 cc of physiologic salt solution, and shake, do this twenty times. Then add twenty portions of 0.2 cc each shaking after each addition of 0.2 cc. Add portions of 0.4 cc of physiologic salt solution until the total quantity measures 8 cc. The optimum dilution must be determined for each antigen. I determine the optimum dilution by titration as is shown in Table I.

According to the results shown in Table I 1:80, I would accept as the optimum dilution.

COMPLEMENT SERUM

Complement serums¹⁰ from different guinea pigs do not always give identical results. Therefore, I select the complement serums. Each serum is titrated against the negative control serum and against the positive control serum, as is shown in Table II. Satisfactory serums are used and unsatisfactory serums are discarded.

Table II shows three good serums and three poor serums. Serums Nos 1, 4 and 6 do not give positive results with the Wassermann test but show good fixation in the presence of syphilitic human serum. Serum No 2 has poor hemolytic power, Serum No 3 gives a positive result with the Wassermann test, and Serum No 5 shows poor fixation in the presence of syphilitic human serum. Serums Nos 2, 3 and 5 are rejected and Serums Nos 1, 4 and 6 are used in the test.

The satisfactory serums are pooled and preserved. Complement serum can be kept frozen¹¹ or salted¹². I prefer to add 720 mg of sodium chloride per 20 cc of guinea pig serum, and keep it at a temperature of about 1° C. For use, serum so salted is diluted 1:5 with distilled water and any further diluting is done with physiologic salt solution (0.9 per cent).

TABLE II
COMPLEMENT TITRATION

NEGATIVE CONTROL SERUM PRECISION METHOD							POSITIVE CONTROL SERUM PRECISION METHOD							
NO OF SERUM	READINGS						RESULTS	READINGS						RESULTS
	ANTIGEN TUBES			CONTROL TUBES				ANTIGEN TUBES			CONTROL TUBES			
	1	2	3	1	2	3		1	2	3	1	2	3	
1	0	0	±	0	0	±	good	±	+	+	0	0	±	good
2	0	±	+	0	±	±	poor	+	+	+	0	±	+	poor
3	0	+	+	0	0	±	bad	+	+	+	0	0	±	↓
4	0	0	±	0	0	±	good	+	+	+	0	0	±	good
5	0	0	±	0	0	±	good	0	±	+	0	0	±	poor
6	0	0	±	0	0	±	good	+	+	+	0	0	±	good

PRIMARY INCUBATION

Fixation of complement in the Wassermann test is not instantaneous. Elsewhere^{13, 14, 15} I reported that fixation of complement is not always completed in five or six hours, and that the most suitable temperature seems to be near the freezing point, about 1° C.

RED BLOOD CORPUSCLES

As red blood corpuscles in the hemolytic system, I use those of man, because it is more convenient. The corpuscles are washed three or four times

with physiologic salt solution, and a 1:40 (2.5 per cent) suspension of the corpuscles is made in physiologic salt solution. The last centrifuging is done for ten minutes at fairly high speed to pack the corpuscles well.

HEMOLYTIC AMBOCEPTOR

As hemolytic amboceptor, I use the blood serum of rabbits that have been immunized against human blood corpuscles, which have been well washed with physiologic salt solution. The rabbit serum is heated to about 56° C for thirty minutes, and is mixed with an equal volume of glycerol No. 1. In the precision method and in the routine method described elsewhere,¹⁶ I use 1.5 unit of amboceptor as titrated in the presence of 0.2 cc of 1:10 dilution of complement serum. Titration of the hemolytic amboceptor is done as follows: into a suitable test tube rack put eight suitable test tubes, designated as Tubes Nos. 1, 2, 3, 4, 5, 6, 7 and 8, as shown in Table III. Into each tube put 0.2 cc of physiologic salt solution. Dilute the amboceptor serum 1:625 with physiologic salt solution, and add 0.2 cc of the diluted amboceptor to Tube No. 1. Mix the contents of Tube No. 1 and transfer 0.2 cc from Tube No. 1 to Tube No. 2 and so continue making dilutions until each tube contains 0.2 cc of diluted amboceptor. To each tube add 0.2 cc of 1:40 suspension of washed blood corpuscles. Put the tubes into the incubator at 37.5° C for thirty minutes. While the corpuscles are being sensitized, shake the tubes at intervals of ten minutes and mix the complement and 50 per cent glycerol as follows: into a small flask or a large test tube put 2.5 cc of complement serum diluted 1:10, 2.5 cc of 50 per cent solution of glycerol and 5 cc of physiologic salt solution. After the red corpuscles have been sensitized for thirty minutes, add 0.8 cc of the complement-glycerol mixture, shake the tubes well, and put them into a water-bath at 37.5° C for thirty minutes, shaking them at intervals of ten minutes. After thirty minutes in the warm bath let them stand at room temperature for thirty minutes without shaking and read the results. The last tube which shows complete hemolysis contains one hemolytic

TABLE III
TITRATION OF HEMOLYTIC AMBOCEPTOR

NO. OF TUBE	1	2	3	4	5	6	7	8
Amboceptor, 0.2 cc diluted	1:125	1:25	1:50	1:100	1:200	1:400	1:800	1:1600
Corpuscle suspension, cc	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
In the incubator at 37.5° C for thirty minutes								
Complement serum, 1:10, cc	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Physiologic salt solution, cc	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Glycerol 50 per cent, cc	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
In the water bath at 37.5° C for thirty minutes and at room temperature for thirty minutes								
Read results								
Results	0	0	0	0	0	0	±	+

unit of amboceptor. Table III shows complete hemolysis at a dilution of 1:400 and only slight hemolysis at a dilution of 1:800. Tube No. 6 (1:400) contains about one hemolytic unit of amboceptor.

READING RESULTS

Results are read and reported in terms of fixing units per cubic centimeter of human serum tested. In previous reports, ¹⁵⁻¹⁷ described the fixing unit as the smallest quantity that will completely fix the complement contained in 0.1 cc of guinea pig serum, using portions of 1 cc of each ingredient in the test. If 1 cc of a 1:108 solution of the human serum contains the smallest quantity of serum which completely fixes the complement contained in 1 cc of a 1:10 dilution of guinea pig serum, the human serum is said to contain 108 fixing units per cc. In daily work, I use 0.2 cc quantities of all ingredients and the highest dilution of the human serum, which completely fixes the test dose of complement serum, corresponds to the number of fixing units per cc as is shown in Tables IV and IV A.

SENSITIVENESS

I have compared this method with the Kolmer modification of the Wassermann test, with the Kahn precipitation test and with the Meinicke precipi-

TABLE IV
READING RESULTS BY THE ROUTINE METHOD

NO OF SERUM	NO OF TUBE	CONTROL TUBE	ANTIGEN TUBES						RESULTS
			1	2	3	4	5	6	
			1:4	1:4	1:12	1:36	1:108	1:324	
1	Readings	0	+	+	0	0	0	0	12
2	Readings	0	+	+	+	+	0	0	36
3	Readings	0	+	+	+	+	+	0	108

TABLE IV A
READING RESULTS BY THE PRECISION METHOD

NUMBER OF SERUM	DILUTION OF SERUM	READINGS						UNITS PER C C
		ANTIGEN TUBES			CONTROL TUBES			
		1	2	3	1	2	3	
1	1 4	0	±	+	0	0	±	4
2	1 4	±	+	+	0	0	±	8
3	1 50	0	±	+	0	0	±	50
4	1 50	±	+	+	0	0	±	100

tation test and in previous reports, ¹⁸⁻²⁰⁻²¹ I showed that I find my method more sensitive than the other methods mentioned.

METHODS

I have the routine method and the precision method. Each can be used with blood serum or with cerebrospinal fluid. Larger quantities of cerebrospinal fluid are used than of blood serum.

Procedure

Prepare three different glycerol solutions as follows:

Glycerol No. 1—To 500 cc of chemically pure glycerol add 4.5 gm of chemically pure sodium chloride and let it dissolve.

Glycerol No 2—To 100 c c of glycerol No 1, add 50 c c of physiologic salt solution

Glycerol No 3—To 100 c c of glycerol No 1, add 100 c c of physiologic salt solution

Sterilize these three solutions

Human Serum—Put 0.2 c c of clear serum into a test tube and place it into a water-bath at about 56° C for thirty minutes. Remove the tube with the serum from the water-bath, let it cool for a few minutes and add 0.6 c c of glycerol solution No 2. This dilutes the human serum 1:4 and the glycerol content is 50 per cent. Note that the serum is heated before the glycerol is added. I find that when raw serum is mixed with glycerol, and then heated, the mixture frequently becomes anticomplementary in a few days, while this does not happen as a rule when the serum alone is first heated. Any further diluting is done with glycerol Solution No 3, always keeping the percentage of glycerol and of sodium chloride uniform.

Antigen—Into a 25 c c graduated cylinder, put 0.1 c c of so-called antigen (alcoholic extract of syphilitic human heart muscle or of normal beef heart muscle to every 100 c c of which 25 mg of cholesterol have been added). Add 0.1 c c of physiologic salt solution and shake, add another 0.1 c c of physiologic salt solution and shake. Repeat this until 20 times 0.1 c c of physiologic salt solution has been added. Repeat twenty times with 0.2 c c portions of physiologic salt solution after which add 0.4 c c portions of physiologic salt solution until the total quantity measures 8 c c. Briefly, it is done as follows: 20×0.1 c c of physiologic salt solution plus 20×0.2 c c of physiologic salt solution plus 5×0.4 c c of physiologic salt solution equals about 8 c c.

Complement Serum—The complement serums have been selected, they have been pooled and 360 mg of chemically pure sodium chloride have been added for every 10 c c of serum. The salted complement serum is kept in the ice cold water-bath.

Add 1 c c of salted complement serum to 4 c c of sterile distilled water and add 5 c c of physiologic salt solution. This dilutes the complement serum 1:10 and the sodium chloride concentration is 0.9 per cent.

At this stage, set up the test, after that wash the corpuscles, dilute the hemolytic amboceptor, titrate and adjust the hemolytic system.

Blood Corpuscles—Wash human blood corpuscles three or four times with physiologic salt solution, allowing the centrifuge to run ten minutes the last time to pack the corpuscles well. Read the volume of packed corpuscles and make a 1:40 (2.5 per cent) suspension in physiologic salt solution.

Hemolytic Amboceptor—The hemolytic amboceptor is the blood serum of a rabbit which was immunized to human blood corpuscles. After bleeding the rabbit, the serum was centrifuged until clear, it was heated to 56° C for thirty minutes and was mixed with an equal volume of glycerol No 1. At the preliminary titration, it was found that 0.2 c c of 1:400 dilution contained about 1 hemolytic unit.

Add 0.1 c c of amboceptor serum to 35 c c of physiologic salt solution and mix well. Take three test tubes, into the first tube put 0.2 c c of diluted

amboceptor, into the second tube put 0.133 c.c. and into the third tube put 0.1 c.c. of diluted amboceptor. With physiologic salt solution bring all volumes up to 0.2 c.c. per tube. To each tube add 0.2 c.c. of corpuscle suspension and put the tubes into the incubator at 37.5° C. for thirty minutes shaking them at intervals of about ten minutes.

Into a suitable test tube put 0.8 c.c. of diluted complement serum, 1.6 c.c. of physiologic salt solution and 0.8 c.c. of glycerol solution No. 3. Mix well and add 0.8 c.c. to each tube of sensitized corpuscles. Put the tubes into the warm water bath at 37.5° C. for thirty minutes shaking them at intervals of ten minutes. Remove the tubes from the water bath, let them stand quietly at room temperature for thirty minutes and read the results. If the corpuscles in the first and second tubes are dissolved with only slight hemolysis in the third tube the dilution is correct; every 0.2 c.c. contains 15 hemolytic units and that is the test dose. Before adding to the test proper mix equal parts of corpuscle suspension and diluted hemolytic amboceptor and sensitize for thirty minutes.

THE TEST (ROUTINE METHOD)

For each human serum to be tested put six suitable test tubes into a suitable test tube rack and designate the tubes as Nos. 1, 2, 3, 4, 5 and 6. Let Tube No. 1 be the control tube and Tubes Nos. 2, 3, 4, 5 and 6 the antigen tubes. Into each of Tubes Nos. 3, 4, 5 and 6 put 0.2 c.c. of 50 per cent solution of glycerol (Solution No. 3). Into Tubes Nos. 1 and 2 put 0.2 c.c. of human serum diluted 1:4 and into Tube No. 3 put 0.1 c.c. of human serum diluted 1:4. Mix the contents of Tube No. 3 and transfer 0.1 c.c. of the contents to Tube No. 4; mix the contents of Tube No. 4 and transfer 0.1 c.c. to Tube No. 5; mix and transfer 0.1 c.c. to Tube No. 6; mix and discard 0.1 c.c.

The first dilution being 1:4 and the other dilutions being multiplied by 3, the dilutions in the different tubes are as follows: Control tube 1:4, antigen tubes 1:4, 1:12, 1:36, 1:108, 1:324. These are the dilutions I mostly use for serums, but other dilutions may be used without changing the results.

Add 0.2 c.c. of physiologic salt solution to each control tube and 0.2 c.c. of diluted antigen to each antigen tube and shake them. Add 0.2 c.c. of complement serum diluted 1:10 and 0.2 c.c. of physiologic salt solution to each tube (the complement serum may be diluted 1:20 and 0.4 c.c. may be added to each tube when human serums only are to be tested, but in testing cerebrospinal fluid these must be added separately as will be seen later). Shake the tubes well and put them into ice cold water for at least five hours shaking them well about two hours after putting them into the cold water.

Wash blood corpuscles and prepare a 1:40 (2.5 per cent) suspension in physiologic salt solution.

Titrate the hemolytic amboceptor in the presence of 0.2 c.c. of complement serum diluted 1:10, 0.2 c.c. of Glycerol Solution No. 3 and 0.2 c.c. of the 2.5 per cent suspension of washed corpuscles as described above. The smallest quantity of amboceptor which dissolves the corpuscles in one hour is called one hemolytic unit. Dilute the hemolytic amboceptor so that 0.2 c.c. contains 15 hemolytic units.

THE PRECISION METHOD

Prepare the human serum, the antigen and the hemolytic system as for the Routine Method. Prepare three different dilutions of complement serum, for serum dilute the complement serum 1:10, 1:20, 1:40 and use 0.4 c.c. as the test dose. For cerebrospinal fluid dilute the complement serum 1:5, 1:10, 1:20 and use 0.2 c.c. as the test dose.

THE PRECISION METHOD FOR SERUM

Put six suitable test tubes into a suitable test tube rack and designate them as Tubes Nos 1 2 3 1', 2' and 3. Into each tube, put 0.2 c.c. of human serum diluted 1:4. Add 0.2 c.c. of diluted antigen to each of Tubes Nos 1, 2 and 3 and 0.2 c.c. of physiologic salt solution to each of Tubes Nos 1', 2' and 3. To each of Tubes Nos 1 and 1' add 0.4 c.c. of complement serum diluted 1:10 to each of Tubes Nos 2 and 2' add 0.4 c.c. of complement serum diluted 1:20 and to each of Tubes Nos 3 and 3' add 0.4 c.c. of complement serum diluted 1:40. Shake the tubes well and finish the test as by the Routine Method. The set up is shown in Table VII.

CEREBROSPINAL FLUID BY THE PRECISION METHOD

Heat 15 cc of cerebrospinal fluid to 56° C for thirty minutes and set up the test as shown in Table VIII.

NEGATIVE CONTROL SERUM

Save the human serums which give negative results, heat them to about 56° C for thirty minutes and add an equal volume of Glycerol No 1 Pool the serums

TABLE VII
SET UP FOR SERUM BY THE PRECISION METHOD

NO OF TUBE	ANTIGEN TUBES			CONTROL TUBES		
	1	2	3	1	2	3
Human serum diluted 1:4 cc	0.2	0.2	0.2	0.2	0.2	0.2
Physiologic salt solution cc				0.2	0.2	0.2
Diluted antigen cc	0.2	0.2	0.2			
Complement serum diluted 1:10 cc	0.4			0.4		
Complement serum diluted 1:20 cc		0.4			0.4	
Complement serum diluted 1:40 cc			0.4			0.4

Finish the test as by the routine method

TABLE VIII
"SET UP" FOR CEREBROSPINAL FLUID BY THE PRECISION METHOD

NO OF TUBE	ANTIGEN TUBES			CONTROL TUBES		
	1	2	3	1	2	3
Cerebrospinal fluid cc	0.2	0.2	0.2	0.2	0.2	0.2
Glycerol 50 per cent, cc	0.2	0.2	0.2	0.2	0.2	0.2
Physiologic salt solution cc				0.2	0.2	0.2
Diluted antigen, cc	0.2	0.2	0.2			
Complement serum diluted 1:5, cc.	0.2			0.2		
Complement serum diluted 1:10 cc		0.2		0.2	0.2	
Complement serum diluted 1:20 cc						0.2

Finish the test as by the routine method

POSITIVE CONTROL SERUM

Save the human serums which give very strongly positive results, heat them to about 56° C for thirty minutes and add an equal volume of Glycerol No 1 to each. Leave them in the refrigerator for at least a month and test them again, reheating the portion to be tested. Some serums become anti-complementary to such an extent that they are unfit, these must be discarded. Pool the suitable serums, place the pooled serum in ice cold water or at lower temperature for a week and titrate it. When using it as control serum, I dilute it with Glycerol Solution No 3 so that the test dose (0.2 c.c.) contains about three fixing units. I always reheat that portion of negative and of positive control serum to be used in connection with the Wassermann test.

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A COMPARISON OF FOUR BLOOD SUGAR METHODS

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IN A COMPARATIVELY short period of time quantitative estimation of sugar in the blood has attained a position of definite importance in the field of clinical diagnosis and medical research. The value of these estimations lies not only in the differential diagnosis of glycosuria but the determinations also serve as a guide in scientific treatment.

As it is not always possible or practical to make a venipuncture the present investigation was undertaken to test the relative reliability in the routine laboratory of two micromethods using only 0.1 cc. of blood for the determination of blood sugar, as compared with the methods at present used in the Virginia Mason Clinical Laboratory, i.e., the Myers-Bailey¹ modification of the Lewis-Benedict method and the Folin-Wu method. Modifications of the latter method, which give slightly lower results, have been reported by both Benedict² and Folin³ in recent years; however the above methods have been found to give consistent results corresponding with clinical findings.

For the blood sugar determination in cases where two cc. or more of blood could not be readily obtained by venipuncture (as in infants, small children, individuals with small or invisible veins and in patients where frequent determinations may be necessary as in diabetic coma) a simple micromethod using only 0.1 cc. of blood which can easily be obtained from ear or finger puncture would be a distinct advantage. The two methods tried were a micro modification of the Folin-Wu method after Gibson⁴ and Folin's new micromethod.⁵

The procedure used in each of the four methods followed very closely the method described by the respective author.

EXPERIMENTAL

The comparison was made on 10 normal individuals and 24 patients with glycosuria. The macro estimations were made on blood from venipuncture and the micro estimations were made on blood from ear puncture, both specimens being taken simultaneously by different technicians. Folin's new 0.1 cc. pipettes, as sold by Eimer and Amend, No. 1, were used for both micromethods. Standardized 5 cc. or 1 cc. serologic pipettes were used in the macromethods. The solutions for all the methods were made up according to directions laid down by the authors in their original publications.

Fasting blood sugars were run by all four methods on 10 individuals who were normal as far as their blood sugar was concerned. Estimations were made using the new Folin micromethod both upon blood from ear puncture and upon a 0.1 cc. portion of the venous blood used for the Myers-Bailey and

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Folin-Wu methods The results in these cases checked very closely, with the exception of the Gibson micro-modification of the Folin-Wu method Folin's new micromethod checked with the two macromethods when the determination was made upon the same sample of venous blood, on blood from ear puncture the values were slightly lower The results are given in Table I

TABLE I
COMPARISON OF BLOOD SUGAR DETERMINATIONS ON NORMAL INDIVIDUALS
(Milligrams Per 100 c c of Blood)

CASE NO	MYERS BAILEY	FOLIN WU	FOLIN NEW MICRO VENOUS	FOLIN NEW MICRO	GIBSON MICRO FOLIN WU
1	154	155	154	150	161
2	110	97	102	92	88
3	112	115	112	108	100
4	97	95	94	90	85
5	125	128	128	124	103
6	102	100	103	97	94
7	117	110	114	110	100
8	110	97	110	110	94
9	98	100	101	96	88
10	123	117	125	125	105

In a sugar tolerance curve performed on a patient with exophthalmic goiter, Table II, the Myers-Bailey method gave a slightly higher curve than did any of the other methods The values for the fasting blood sugar by the first methods gave practically the same results

TABLE II
COMPARISON OF BLOOD SUGAR DETERMINATIONS IN SUGAR TOLERANCE CURVE
(Milligrams Per 100 c c of Blood)

TIME	MYERS BAILEY	FOLIN WU	FOLIN NEW MICRO	GIBSON MICRO FOLIN WU
Fasting	83	91	85	75
$\frac{1}{2}$ hour	250	230	244	210
1 hour	250	249	243	210
2 hours	210	188	200	175
3 hours	153	126	120	110

Table III gives the results on a series of 24 patients under treatment for diabetes and showing glycosuria In some cases the blood was taken after fifteen hours fasting, in others, the specimen was taken one hour after lunch Blood-sugar estimations were made by the four methods being studied

In the first five cases single tubes with four c c of filtrate were used for Folin's new micromethod and the results were low, in Cases 4 and 5 the method was rerun on 2 c c of filtrate and checks were obtained with the other methods In subsequent cases of the series three tubes were set up in each case instead of one Into the first tube was pipetted 4 c c of the clear filtrate, into the second tube 2 c c of filtrate and 2 c c of distilled water, and into the third tube 1 c c of filtrate and 3 c c of distilled water The three tubes were run at the same time Accurate readings of over 200 milligrams are practically impossible with the first tube and the same is true of over 400 milligrams with the second tube There is plenty of filtrate for this procedure, and it not

TABLE III
COMPARISON OF BLOOD SUGAR DETERMINATIONS ON DIABETIC CASES
(Milligrams Per 100 c.c. of Blood)

CASE NO	MYERS BAILEY	FOLIN WU	GIBSON MICRO FOLIN WU	FOLIN NEW MICRO	REMARKS
1	484	475	510	206	Fasting
2	460	450	467	225	Fasting
3	420	468		244	Fasting
4	374	374	426	240	Fasting
4a				367	Rerun with 2 c.c. filtrate
5	367	380	378	248	Fasting
5a				355	Rerun with 2 c.c. filtrate
6	300	312	350	278	One hour after lunch
7	300	307	290	300	Fasting
8	187	185	214	173	Fasting
9	180	162	181	190	One hour after lunch
10	235	214	272	200	Fasting
11	211	185	200	222	One hour after lunch
12	300	280	250	230	Fasting
13	187	185	166	181	Fasting
14	166	170	162	166	Fasting
15	154	155	161	150	Fasting
16	284	296	270	278	Fasting
17	120	115	112	125	Fasting
18	600	590		664	Coma
19	600		-	666	Coma
20	194	--	-	170	Fasting
21	88	85	-	80	Fasting
22	200	193	-	177	Fasting
23	166	152	-	160	One hour after lunch
24	230	228	--	212	Fasting

Only one tube containing 4 c.c. of filtrate was used in the first determinations by the new Folin micromethod. In all subsequent determinations three tubes were set up simultaneously.

only saves a great deal of time but also removes the doubt from determinations which are not being checked by some other method and are near the limit of accuracy of the new Folin method

The Folin Wu method and the Myers Bailey modification of the Lewis Benedict method checked very closely, many of the slight variations being less than experimental error of the methods. The new Folin micromethod paralleled the two macromethods giving values averaging a few milligrams lower than the other two methods. However the differences were not sufficiently great to be significant in clinical interpretation. In Cases 18 and 19 difficulty was experienced in securing sufficient blood by venipuncture for the macromethods, so that the higher values of the micromethod are probably the more accurate. The Gibson micro-modification of the Folin Wu method showed greater and less consistent variations and consequently was discarded.

SUMMARY

1 Folin's new micromethod for blood sugar determination gave results in excellent agreement with the older macromethods on both normal and diabetic blood.

2 Folin's new micromethod checked very closely with the Folin Wu method and Myers Bailey modification of the Lewis Benedict method when the determination was made on the same sample of venous blood. On blood

from ear puncture the values were a few milligrams lower than on venous blood drawn at the same time

3 Setting up three tubes with varying amounts of filtrate in place of one in Folin's new micromethod was found not only to save a great deal of time in the clinical laboratory, but gives assurance to the determinations, removing the doubt from those determinations showing values near the limit of accuracy of the method

4 The Gibson micro-modification of the Folin-Wu method gave results which were considered to have too great and too inconsistent a variation from the average of the other three methods

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A NEW TYPE OF TISSUE CRUSHER*

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THE usual method of culturing surgically removed tissue, or tissue removed in a sterile manner, by grinding in mortars, is tedious and offers sources for contamination. I have employed this method for years, using sand as the abrasive. This is fairly reliable as long as the material to be ground is relatively soft and easily disintegrated, such as liver tissue, but when dense fibrous tissue, such as a uterine fibroid tumor, is to be ground, it is almost impossible to macerate it in a mortar without its becoming contaminated.

Rosenow,† in 1914, reported on the use of two methods, or appliances, which effectively eliminated the usual sources of contamination. One of these methods consisted of grinding the tissue in a sterile air chamber which contained a small meat chopper and a mortar. The tissue was ground either in the mortar alone or, if it was particularly tough or large in size, it was first passed through the meat chopper and then ground in the mortar. The hand was placed in a sterilized glove, the outer surface of which formed part of the lining of the sterile chamber. However, this method was rather cumbersome and bulky when numerous cultures were required. At the same time, Rosenow reported on an all-metal device by which the tissue that was to be emulsified was forced by means of a threaded hollow plunger through numerous small holes of a disk. This method, although it eliminates sources of contamination,

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†Rosenow, E C The Newer Bacteriology of Various Infections as Determined by Special Methods Jour Am Med Assn 1914 July 903-907

also has its practical drawbacks, the small holes are hard to clean and there is a tendency for the tissue to be forced backward along the screw threads.

Several years ago, Rosenow used another method for handling extracted teeth. He caused to be constructed a small hollow metal cylinder and a metal plunger which fitted it. He placed the extracted tooth in the cylinder, and by pounding on the plunger the tooth was crushed. From the debris, the pulp or apex could be picked out for culture.

Using this instrument, I attempted to crush tissue but was unable to accomplish anything because of the resiliency of the tissue. I then thought of using a grinder resembling a pestle working in a mortar but employing an eccentric axis. The method was similar to that used in grinding eye glasses. However, the tissue would work to the side away from the grinding area.

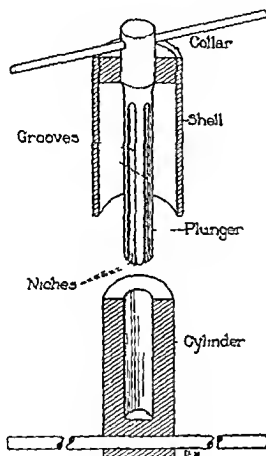


Fig 1—Tissue crusher

Then the idea occurred to me that if the outlets for the crushed tissue were made on the periphery of the plunger instead of in the cylinder head, there would be only one avenue of escape for the tissue. This, with several modifications to overcome technical difficulties that arose led to the development of the present instrument (Fig 1). An instrument of the size described in this article has been very satisfactory for the majority of the tissues from which cultures have been made.

The instrument consists essentially of two parts: the first part is a hollow cylinder; the second part is a combined plunger and shell which fit respectively into the cylinder and over the outside of it. The cylinder is made of a bar of monel metal one inch in diameter and two and a fourth inches long in which is drilled a hole three eighths of an inch in diameter and one and three fourths inches deep. This leaves the base thick enough for a three sixteenth inch hole to be drilled crosswise through it. A steel rod is pushed through

this hole for leverage. The plunger consists of a solid, steel, nickel-plated cylinder three and a fourth inches long and three-eighths of an inch in diameter, it fits snugly, yet slides easily into the hollow cylinder. The steel shell that has been mentioned is two and an eighth inches long, $1\frac{5}{32}$ inches wide over all and approximately one-thirty-second of an inch thick, it screws onto a collar three-eighths of an inch thick, which is fixed on the plunger so as to leave two and three-eighths inches of the plunger below the lower surface of the collar. On the plunger are four grooves which run lengthwise and which are equidistant from each other, each groove is one-eighth of an inch wide, about one-twenty-fifth of an inch deep, two and a fourth inches long, and begins one-eighth of an inch from the lower end of the plunger. On this lower one-eighth of an inch of the plunger are twelve small notches made with a three-cornered file, they are about one-thirty-second of an inch wide and one-thirty-second of an inch deep. As seen in cross-section, on the lower face of the plunger, they are approximately equidistant from each other, but they are so placed that each three notches converge and lead into each of the four long vertical grooves just described. At the opposite end of the plunger is a crosswise hole an eighth of an inch in diameter similar to the hole in the base of the cylinder, through this hole a rod is inserted for leverage.

After the instrument has been sterilized in the autoclave, it is used as follows. A piece of tissue to be cultured is inserted in the cylinder, with sterile forceps or scissors, and is pushed toward the bottom, all pockets are avoided if possible. The plunger is then inserted as far as it can be shoved easily. The sterile towel in which the instrument was wrapped is wrapped especially around the lower edge of the shell and the entire instrument is then placed in a vise. Pressure is made on the top of the plunger and base of the cylinder, and as the vise is screwed shut the tissue is forced through the twelve fine grooves, up along the four parallel larger grooves and into the base of the shell. The vise is then loosened, the two metal rods are inserted into the holes in the cylinder and plunger and then rotated, if necessary, to loosen the plunger from the cylinder. The sterile towel is then removed, the plunger, with its shell, is removed from the cylinder after the lower edge of the shell has been flamed, and the macerated tissue is found along the grooves and in the bottom of the cup formed by the shell and the collar on the plunger. With a sterile pipette and salt solution, the macerated tissue is washed from the plunger and diluted in the cup and from there it is pipetted into the desired culture mediums. It is advisable to tilt the vise backward slightly from the usual horizontal plane so that the tissue juices will gravitate to the base of the shell.

The plunger, from the lower end to the lower surface of the collar, is made five-eighths of an inch longer than the hole in the cylinder, so that there is space provided for the macerated tissue when the plunger is driven home. The shell is made large enough to provide a clearance of about three-sixty-fourths of an inch between it and the cylinder. When the plunger is driven home, the lower edge of the shell is one and one-eighth inches from the base of the cylinder. This provides ample space to take hold of the cylinder without contaminating the lower edge of the shell. It is desirable to mark on the

cylinder the position of the lower edge of the shell when the plunger is in the cylinder as far as possible so that undue pressure shall not be made after the plunger is at the bottom of the shaft. Otherwise, it is likely to be bent. If a very sinewy piece of tissue seems to resist crushing it sometimes can be crushed by closing the vise in jerks and waiting a moment between each partial turn of the vise handle. Originally, it was thought advisable to hollow the upper end of the plunger and insert a ball bearing to provide rotation of the plunger while closing the vise, but so far this has not been needed. The twelve small nitches in the plunger can be filed to snit the tissue to be cultured. However, I have found that nitches which are very small give very fine maceration but require tremendous power to close the vise. Under these conditions the plunger often is bent, this makes much more work and sometimes the tissue is contaminated in attempts to remove the bent plunger. For this reason, also the plunger is made of steel, since it is harder than monel metal. The nickel plating is used to prevent rusting.

Crushing the tissue evidently does not kill the organisms since resected ulcers and diseased gall bladders cultured in this manner may yield just as good a culture of pathogenic organisms as that obtained with the mortar and pestle. Moreover, in cultures from tissue macerated with the mortar and pestle the incidence of organisms indicating contamination was higher than that when the tissue crusher just described was used. I hope that further investigation with known cultures will confirm the fact that the tissue crusher lessens the chances for contamination.

A SIMPLE AND ACCURATE HEMATOCRIT*

BY M. M. WINTROBE, M.D., NEW ORLEANS, LA

THE determination of cell volume—the volume of packed red cells in a given sample of blood, is a most valuable procedure in the diagnosis and differentiation of the anemias. From the red cell count, hemoglobin and cell volume, the volume index and the saturation index can be determined^{1, 2} and the volume of the average red corpuscle, as well as its hemoglobin content, calculated.³ The determination of cell volume has not been generally adopted as a laboratory diagnostic procedure principally because of lack of appreciation of the information to be derived and secondly, because a simple and yet accurate hematocrit has not been available. A hematocrit which meets these requirements is here described.

The instrument (Fig. 1, A) is a narrow glass tube 11 cm. in length of even bore (3 mm. inside bore), and with a flat bottom. A centimeter millimeter scale, commencing at the level of the inside bottom of the hematocrit, is etched on the glass.†

*From the Department of Medicine, Tulane University, School of Medicine, New Orleans.
Received for publication July 1, 1929.

†The instruments have been made for us by Corning Glass Works, Corning, N. Y.

A NEW RAPID PARAFFIN METHOD FOR TISSUE SECTIONS*

BY IDA M. VILKOMERSON, NEW YORK, N. Y.

IN VIEW of the fact that an early report on tissues often is of importance, a rapid yet efficient method for running through sections is desirable. At best, present paraffin methods consume three days. Therefore to hasten such work, a series of experiments were made during which the following procedure was found to be very satisfactory, tissues being ready for diagnosis within four hours after the fresh specimen had been received.

The tissue segments should not be more than 2 mm in thickness. It is advisable to use throughout a wide-mouthed bottle to facilitate transfer of tissue. The specimen is first placed in the following solution:

Absolute methyl alcohol	60 cc
Acetone (C. P.)	100 cc
Iodine crystals	1 to 2 crystals
Anhydrous copper sulphate	$\frac{1}{2}$ inch layer

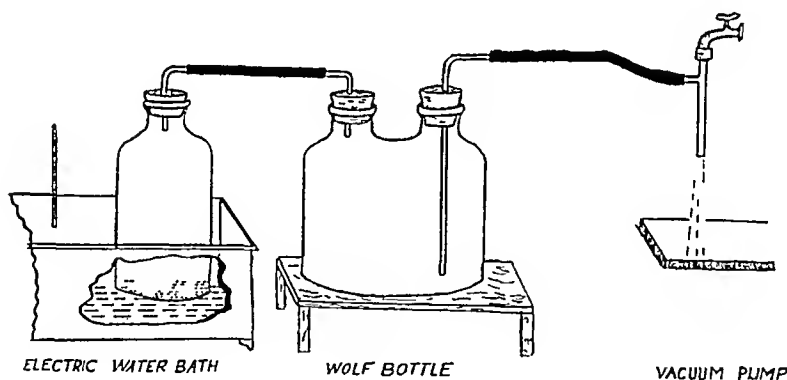


Fig. 1

The combination of absolute methyl alcohol and acetone is ideal, as it fixes and dehydrates at the same time, whereas in all other methods this process consists of two separate steps requiring from several hours to two days. Addition of a few iodine crystals increases the penetrating power of the solution. The half-inch layer of anhydrous copper sulphate at the bottom of the bottle removes any remaining traces of water. The copper sulphate is covered with several ply of gauze, upon which the tissue is placed for one and one-half hours. It is then transferred to chloroform for thirty minutes, then to a mixture of equal parts of chloroform and paraffin for fifteen minutes.

The tissue is next placed in clean melted paraffin (55°C) for fifteen min-

*From the Bendiner and Schlesinger Research Laboratory, New York.
Received for publication July 31, 1929.

utes, every trace of chloroform being removed, and then transferred to a bottle containing filtered paraffin to which is attached a vacuum apparatus (see Fig 1) The tissue remains in vacuo in the paraffin for fifteen minutes For this purpose an electric water bath (56° C) may be used This can readily be brought near a sink with a vacuum pump attachment and connected to a Wolf bottle to prevent back flow of water or it may be used with vacuum piped system In using the paraffin with vacuum, there are several advantages over other paraffin methods usually requiring several hours in the oven (1) By the vacuum method there is very much better infiltration with paraffin, (2) there is considerably less exposure to heat thus preventing shrinkage of tissue (3) the vacuum also helps to draw out the last traces of chloroform and therefore insures perfect imbedding, (4) much time is saved It is important that the temperature of the water bath should not exceed 1° C above the melting point of the paraffin used

After this treatment the infiltrated tissue is handled in the usual fashion but only ten minutes drying in an oven at 37.5° C is necessary, if excess water is wiped off the edge of the slide It was found unnecessary to use albumin to cause the sections to adhere to the slides if the latter were cleansed with acid alcohol to remove grease

SUMMARY

Running through of tissue sections

SOLUTION 1			
Absolute methyl alcohol	- - - - -	60 cc	} 1½ hours
Acetone (C P)	- - - - -	100 cc	
Iodine crystals	- - - - -	1 to 2 crystals	
Anhydrous copper sulphate	- - - - -	½ inch layer	
SOLUTION 2			
Chloroform	- - - - -	-	½ hour
SOLUTION 3			
Chloroform paraffin	- - - - -	-	¼ hour
SOLUTION 4			
Paraffin (plain)	- - - - -	-	¼ hour
SOLUTION 5			
Paraffin with vacuum	- - - - -	-	¼ hour
Imbed	- - - - -	-	
			2¾ hours

CONCLUSION

By this rapid method tissues can be fixed dehydrated imbedded, cut, and stained in from three and a half to four hours If attention is given to all details, it is simple and thoroughly efficient Sections prepared by this procedure keep well and may be filed for future study

THE VAN DEN BERGH REACTION*

A COMPARISON OF TECHNIQS

BY HARRY SHAY, M D, AND EUGENE M SCHLOSS, M D, PHILADELPHIA, PA

IN 1918,¹ van den Bergh introduced his method for determining the presence of bile pigment in body fluids. No one can deny the importance of this reaction in recent studies of jaundice and the stimulus it gave to renewed investigation of many liver conditions. A method for the detection of bile pigment far more delicate than any previously suggested, it has the added advantage of being specific for bilirubin. This specificity readily establishes its superiority over the icterus index, inasmuch as it is not quantitatively altered by hemolysis, lipemic opalescence,² or the presence of other pigments, such as lutein or carotin. The reaction differs also from all other methods of estimating the bilirubin content of body fluids, in its value in indicating the obstructive and nonobstructive types of bilirubin. The theory upon which this qualitative differentiation is based is described in papers by McNee³ and by Bockus and Shay⁴ and need not be entered into here.

We wish to present observations on some modifications of technic which, though far superior to the original methods, have, for some reason, not come into general usage. We would call especial attention to the modifications suggested by Lepehne⁵ for the qualitative reaction and by Thannhauser and Andersen⁶ for the quantitative. Greene, Snell and Walters⁷ mention the superiority of the latter but do not, we believe, sufficiently stress it.

THE QUALITATIVE REACTION

The original qualitative technic carried out by mixing equal quantities of blood serum and reagent, makes it difficult for the operator to judge accurately the early production of color, the production of lesser degrees of color, and the end-point of the reaction. In Lepehne's modification, three small tubes are used, in each of which is placed 0.25 c c of blood serum. To Tube I is added 0.2 c c of water, to Tube III, 0.2 c c of diazo reagent. The tubes are set aside for at least fifteen minutes to allow for completion of the reaction in Tube III. At the end of this interval, 0.2 c c of diazo reagent is added to Tube II, and the color, time of appearance of the color, and time of complete reaction are noted, Tubes I and III serving as controls for the negative and completed reactions respectively.

We have graded our reactions according to the time limits suggested by McNee³ but have also adopted the biphasic classification of Feigl and Queirer.⁶ These include

1 *Prompt Direct Reaction* Bluish violet color beginning immediately upon mixing serum and reagent and becoming maximal in thirty seconds

*From the Gastrointestinal Clinic of the Jewish Hospital
Received for publication August 28 1929

2 *Delayed Direct Reaction* Reddish coloration, beginning only after one to fifteen minutes gradually deepening to more violet

3 *Biphasic Direct Reaction* Reddish color appearing at once and either slowly or rapidly deepening to violet

4 *Negative Reaction* No color appearing within fifteen minutes

Utilizing this technic, we cannot agree with Andrewes⁹ or with Hall¹⁰ that the maximum color is not often reached in thirty seconds in direct immediate sera. While it is impossible to present graphic data for the qualitative reaction, we are convinced that the modified technic enables one to classify far more accurately the reactions obtained. It is particularly useful in cases of mild jaundice where the intensity of the color reaction produced is not very pronounced even when complete. Not infrequently definite direct immediate or delayed reactions can be detected where the use of the single tube technic would place them in the indirect or negative group. The superiority of this method lies in the fact that it establishes controls for the two variables in the reaction, namely, the color production and its production time.

THE QUANTITATIVE REACTION

The original quantitative technic is so frequently described in the literature that it is unnecessary to do so here. Van den Bergh from the start realized that this was not an accurate method and has always called it an "estima-

TABLE I

A COMPARISON OF BLOOD BILIRUBIN READINGS BY THE STANDARD VAN DEN BERGH TECHNIC AND BY THE THANNHAUSER AND ANDERSEN MODIFICATION

CASE NO	DIAGNOSIS	Serum		
		VAN DEN BERGH ORIGINAL TECHNIC	THANNHAUSER AND ANDERSEN MODIFICATION	CONFIRMATION OF DIAGNOSIS
1	Carcinoma of tail of pancreas	0.2	0.7	Operation
2	Carcinoma of tail of pancreas	0.3	0.3	Operation
3	Cirrhosis of liver	0.3	0.5	
4	Carcinoma of stomach	0.3	1.0	Operation
5	Chronic cholecystitis	0.3	1.3	Operation
6	Catarrhal jaundice	0.3	1.5	
7	Carcinoma of cecum	0.4	0.5	Operation
8	Cholelithiasis	0.5	0.6	Operation
9	Cholelithiasis	0.5	0.7	Operation
10	Cholelithiasis	0.5	1.7	Postmortem
11	Cholelithiasis	0.5	2.3	Operation
12	Carcinoma of head of pancreas	0.6	3.4	Operation
13	Catarrhal jaundice	0.7	3.2	
14	Catarrhal jaundice	0.7	5.0	
15	Carcinoma of head of pancreas	1.7	4.1	Operation
16	Hemolytic jaundice	2.0	4.6	Operation
17	Carcinoma of head of pancreas	2.7	14.2	Operation
18	Catarrhal jaundice	3.7	12.5	
19	Cholelithiasis	5.0	15.0	Operation
20	Carcinoma of head of pancreas	5.2	17.5	Operation
21	Carcinoma of stomach and liver	6.4	19.3	X ray
22	Catarrhal jaundice	7.7	9.3	
23	Carcinoma of head of pancreas	9.2	30.3	Operation
24	Catarrhal jaundice	10.8	32.0	
25	Catarrhal jaundice	12.8	35.2	
26	Carcinoma of head of pancreas	14.6	26.7	Operation
27	Carcinomatosis	16.6	25.4	Operation
28	Cholelithiasis	19.5	58.0	Operation

All readings in van den Bergh units

tion." The discrepancy in the reaction is due to the fact that some bilirubin is lost in the albuminous precipitate produced in the test. While it is true that the greatest loss occurs with sera giving a strong prompt direct reaction (see Table I), we cannot agree with McNee and Keefe¹¹ that this loss may be disregarded for sera giving a delayed direct reaction. An appreciable difference (133 per cent) by the old and modified techniques, has even been noted in the blood from a case of true hemolytic jaundice (Table I, Case 16).

The modification of Thannhauser and Andersen is superior in that all of the azo-bilirubin can be retained for estimation in the supernatant liquid. This is accomplished by first mixing the test serum and diazo reagent, allowing coupling to become completed, and then precipitating the albumin with alcohol and saturated ammonium sulphate. One can readily see the difference between the old and new techniques by noting the color of the precipitate after centrifuging. In the former especially, if the serum is heavily jaundiced, the precipitate is always definitely yellow, while by the latter method, the precipitate is *white*. The explanation for this difference in reaction may lie in the far greater solubility of azo-bilirubin in alcohol as compared to that of pure bilirubin.

The details of the modified technique are as follows. To 1 c.c. of serum add 0.5 c.c. of diazo reagent. After allowing coupling to take place (we prefer fifteen minutes), add 2.5 c.c. of 95 per cent alcohol and 1 c.c. of a saturated solution of ammonium sulphate. These are mixed and centrifuged. The mixture separates into three layers, a lower clear watery one, a middle white layer of precipitate, and an upper, clear, reddish violet layer containing the azo-bilirubin. The upper layer is removed and compared colorimetrically with a standard. In our comparative studies we used van den Bergh and Muller's cobalt sulphate standard. This is made up by dissolving 2.161 gm. of *anhydrous* cobaltous sulphate in 100 c.c. of distilled water. This solution gives a color corresponding to one unit of bilirubin and is permanent if kept in the dark. Table I shows a comparison of the old and new methods in a series of 28 determinations in a variety of diseases.

TABLE II

SAME AS TABLE I EXCEPT THAT READINGS ARE TAKEN ON THE BLOOD PLASMA INSTEAD OF ON THE SERUM

Plasma

CASE NO	DIAGNOSIS	VAN DEN BERGH ORIGINAL TECHNIC	THANNHAUSER AND ANDERSEN MODIFICATION	CONFIRMATION OF DIAGNOSIS
1	Cholelithiasis	0.3	0.5	Operation
2	Cholelithiasis	0.5	2.1	Postmortem
3	Carcinoma of head of pancreas	1.6	3.7	Operation
4	Carcinoma of head of pancreas	2.5	8.8	Operation
5	Catarrhal jaundice	3.5	9.5	
6	Carcinoma of head of pancreas	3.9	10.0	Operation
7	Catarrhal jaundice	4.5	24.7	
8	Carcinoma of stomach and liver	5.1	15.1	X ray
9	Carcinoma of head of pancreas	9.3	30.5	Operation
10	Catarrhal jaundice	8.5	33.5	
11	Carcinoma of head of pancreas	10.5	25.3	Operation
12	Carcinomatosis	15.8	18.0	Operation

Table II shows a similar difference in readings obtained by using the plasma instead of the serum

We cannot agree with McNee and Keefer¹¹ that the plasma may be utilized equally as well as the serum. In our comparative series as shown in Table III we obtained consistently lower readings with the plasma as compared with the serum when examined simultaneously. It will be noted in this group that in one instance (Table III Case 8) the plasma reading was

TABLE III

A COMPARISON OF THE BLOOD SERUM AND PLASMA READINGS AS OBTAINED BY BOTH METHODS
NOTE THE PRACTICALLY CONSISTENT HIGHER SERUM READINGS OBTAINED BY THE MODIFIED TECHNIQUE

CASE NO	DIAGNOSIS	VAN DEN BERGH ORIGINAL TECHNIC		THANNHAUSER AND ANDERSEN MODIFICATION	
		SERUM	PLASMA	SERUM	PLASMA
1	Cholelithiasis	0.5	0.5	0.7	0.5
2	Cholelithiasis	0.5	0.5	1.7	2.1
3	Carcinoma of head of pancreas	1.7	1.6	4.1	3.7
4	Carcinoma of head of pancreas	2.5	2.5	14.2	8.8
5	Catarrhal jaundice	3.5	3.5	12.5	9.5
6	Carcinoma of head of pancreas	5.2	3.9	17.5	10.0
7	Carcinoma of stomach and liver	6.4	5.1	19.8	15.1
8	Carcinoma of head of pancreas	9.2	9.3	30.8	30.5
9	Catarrhal jaundice	12.8	8.5	35.2	33.5
10	Carcinoma of head of pancreas	14.6	10.5	26.7	25.3
11	Carcinomatosis	16.6	15.8	25.4	18.0

slightly higher than the serum. In this case the plasma diazo mixture was allowed to stand overnight before precipitating with alcohol. We are inclined to believe that the time permitted for coupling to occur may have some effect on the final reading. Investigations regarding this are now going on in this clinic and will be reported if found of any importance.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE M.D. ABSTRACT EDITOR

SPERMATOOA COUNT In the Diagnosis, Prognosis, and Treatment of Sterility, Macomber, D., and Sanders, M. B. New England, J. M. 200 981, 1929

A blood counting chamber and a white blood cell pipette are necessary. The diluent is a solution of five per cent sodium bicarbonate to which one per cent formalin has been added. The bicarbonate dissolves small amounts of mucus and the formalin is added to stop the activity of the spermatozoa. This semen is drawn up to the 0.5 mark on the pipette and then diluted with the bicarbonate formalin solution to the 11 mark. The pipette is then thoroughly shaken to obtain a uniform mixture. A drop is placed on the counting chamber and the spermatozoa are counted in the same manner as in making a white cell count. After the number of spermatozoa in the millimeter square is determined, the following formula is used to compute the number of spermatozoa per cubic centimeters, $\text{Number in millimeter square} \times 10 \text{ (depth)} \times 20 \text{ (dilution)}$ equals the number per cubic millimeter. $\times 1,000$ equals the number per cubic centimeter. The two chief sources of error are in the cases where there is an excess of mucus in the semen and in cases where it is observed in the preliminary examination under the microscope that the numbers are very low. The first may be corrected by diluting 1 cc of semen with 19 cc of the bicarbonate formalin solution, shaking thoroughly and counting without further dilution. The second may be counted directly in the counting chamber without any dilution whatever. The average normal count is 100,000,000 per cc (calculated from 294 cases).

MERCURY Detection of in Skin Discoloration of Skin Due to Mercury, Hollander, L., and Baer, H. L. Arch Dermat & Syph 20 27, 1929

Two samples of the skin were placed on separate watch glasses, and 15 per cent nitric acid was added to each. These watch glasses were then placed on water baths and heated at the boiling temperature of water for two hours. At the end of this time only a few yellow pieces of solid material remained in the clear solutions.

Chlorine was passed into the solutions, for fifteen minutes. To expel excess chlorine, air was passed through the chlorinated solutions until the odor of chlorine was no longer detectable. The solutions were concentrated to 1 or 2 cc and were electrolyzed.

In the electrolysis, platinum wires were employed as anodes and copper wires had a gauge of 30, and the ends which were to be immersed were ground to fine points and then smoothed with emery cloth. The platinum and copper wires were held in place and isolated from each other during electrolysis by means of a piece of rubber. A storage battery was used to furnish a current and a post office box furnished the necessary resistance. Electrolysis was carried out with a current of 15 milliamperes and 1.5 to 2 volts.

The solutions of the two samples of skin were electrolyzed for thirty minute periods at the same amperage and voltage. During the electrolyses, the platinum anodes were immersed to a depth of approximately 0.5 cm, but the copper cathodes were so arranged that only the points were in the solutions. The electrodes were placed as close as possible in the solutions without having actual contact. At the end of thirty minutes the current was shut off, the copper cathodes were removed, washed with water and dried with clean pieces of silk. To the naked eye the points of copper showed a characteristic bleakening. These points were next examined under the microscope.

In the microscopic examination, the cathode points, which rested on glass slides coated with asphaltum, were examined under a magnification of 100 diameters. Illumination was obtained by the use of a Silverman illuminator.

The samples examined showed characteristic deposits of silver and mercury gathered as small globules on the tips of the copper wires. These were in marked contrast to the copper color of wires which had been polished but not subjected to electrolysis, and wires which had been used as cathodes in thirty minute electrolysis of 1 to 2 cc of water acidified with one drop of 15 per cent nitric acid, which were examined simultaneously on the asphaltum coated glass slides.

As a further proof that deposits of mercury had been obtained, these wires were heated at a distance of 2 to 3 cc from the point which had the globules of mercury, by means of a micro bunsen burner. The period of heating varied from 0.5 to 1 minute. The wires were cooled and when reexamined it was found that the bright metallic globules had disappeared. This coincided with the disappearance of mercury from copper wires which had been used in the electrolysis of weak mercuric nitrate solutions ($Hg(NO_3)_2$, H_2O) and have been subjected to a similar treatment.

SPIROCHETES Staining of in Nervous Tissue Kanzler E. *Ztschr f d ges Neurol u Psychiat* 117 171 1928

The section is immersed for thirty minutes in a solution of ammonium bromide and formaldehyde. It is then washed and put in pyridine for fifteen minutes, washed again and immersed for ten minutes in a 0.5 per cent uranin solution. After being washed again the sections are immersed for one hour in a 1.5 per cent silver nitrate solution at from 37 to 40 C, quickly heated in the silver solution over the flame, washed in distilled water, dipped and moved about for from two to four seconds in a solution of silver nitrate soda and ammonia. Then without being washed they are placed for from three to five seconds in a 5 per cent formaldehyde solution. As soon as they have taken on a yellow or yellowish brown color they are placed in distilled water which is changed several times and consecutively in alcohol, beechwood creosote, phenol, xylene and Canada balsam. The spirochetes are stained black whereas the nervous tissue remains unstained.

INDOL Simple Test for Kovacs N. A. *Ztschr f Immunol u Exper Therap* 55 311, 1928

p-dimethylamidobenzaldehyde (Merek) 0.5 gm is dissolved in pure amyl alcohol 70 cc and concentrated hydrochloric acid 25 cc. From 25 to 30 drops of this solution are added to broth cultures of bacteria. After a gentle shaking a violet red color appears if indol is present in the broth.

BLOOD SUGAR The Preservation of Blood for Estimations of Lax H. and Szirmat I. *München med Wchnschr* 76 58 1929

The glycolysis and bacterial destruction of the sugar in diabetic and normal blood are prevented by the addition of 1 per cent sodium fluoride and 0.1 per cent mercuric chloride. With the addition of these reagents the sugar content of the blood remained unchanged even after thirty days storage in the incubator.

UNDULANT FEVER Isolation of Brucella Organism from the Stools Amoss H. L. and Poston, M. A. *J A M A* 99 170 1929

About 1 gm of fresh feces was mixed in 50 cc of sterile isotonic salt solution and shaken for a few minutes to insure thorough suspension. The suspension was filtered through four layers of No. 1 hospital gauze to remove gross particles and centrifuged at half speed for three minutes to throw down other particles and larger bacteria. To the supernatant suspension a sufficient amount of immune serum was added to make the total dilution 1:100 and after shaking the mixture was placed in a 37 C water bath for two hours. The suspension was centrifuged at half speed for five minutes and the supernatant fluid discarded. The precipitate was resuspended in isotonic salt solution, stirred and centrifuged at the same speed again. The supernatant fluid was again discarded and

the procedure repeated twice. Finally the precipitate was spread with a bent glass rod on eosin methylene blue plates, some of which were incubated at 37° C aerobically and others in an anaerobic jar containing 10 per cent carbon dioxide.

Large clear colonies appeared after ninety six hours. These were fished and the organism identified in the usual manner.

In twenty experiments the patient's own serum known to agglutinate *Brucella melitensis* strain 428 of the Hygienic Laboratory was used to concentrate the fecal organisms. In sixteen experiments, the polyvalent antimelitensis serum produced by Mulford was employed in a dilution of 1:100 and of 1:300 with equal success.

It is suggested that, in cases from which the organism has not been recovered from the urine or the blood but in which the patient's serum agglutinates members of the *Brucella* group, either the patient's serum or the corresponding polyvalent or monovalent serum be used. On account of the dilution employed, the small amount of preservative may be disregarded.

HOOKWORM Suitability of Various Bacteria as Food for Hookworm Larvae, McCoy, O R. Am J Hyg 10 140, 1929

The following method was used for the isolation of the ova.

Thoroughly mix up about 25 to 50 gm of freshly passed feces in about 500 cc of water. An electric mechanical stirrer proved very useful for this purpose. The mixture was then washed through a series of copper wire sieves ranging up to a mesh of 100 wires to the inch. The coarse material in the feces was caught in the sieves but the eggs passed through with the filtrate. The filtrate was then allowed to stand in a large sedimenting cone for about one hour while the eggs and other heavy particles settled to the bottom. The supernatant fluid was then poured off and the sediment transferred to a 50 cc centrifuge tube. This sediment was repeatedly washed by centrifuging at a speed of 1,000 revolutions per minute. When the supernatant fluid from the washings became practically clear, saturated salt solution was added to the sediment and the material again centrifuged at the same speed. This time the eggs came to the surface and could be collected by removing the surface film with the open end of a piece of large glass tubing.

ASCARIS On the Use of a Method for the Isolation of Ascaris Eggs from the Soil, Spindler, L A. Am J Hyg 10 157, 1929

A pint or more of soil is collected from a suspected spot by sweeping or lightly scraping the surface of the ground over a large area. In the laboratory the sample is thoroughly crushed and mixed and a representative 5 to 10 gm portion placed in a 50 cc centrifuge tube and treated for an hour with 10 cc of 30 per cent antiformin solution. It is quite imperative, in this process, that the mixture be given frequent thorough stirrings to allow the antiformin to act on every particle of soil. If this is not done many eggs will remain adhering to the soil particles and will not be recovered in flotation. When sufficient time has elapsed for the eggs to become freed from the soil the tubes are filled with sodium dichromate (specific gravity 1.35), thoroughly shaken and then centrifuged at 1,000 r p m for one or two minutes. The eggs are then looped from the surface of the dichromate to a microscope slide by means of a small vial or the open end of a glass tube. They are then counted and classified according to the stages of development to which they have attained.

ARTHRITIS Gland Cultures in Infectious, Poston, M A. J A M A 93 692, 1929

By previous arrangement the necessary mediums and instruments are ready for the preparation of the cultures of the gland immediately after removal. If other work prevents immediate culture, the glands are moistened with sterile isotonic salt solution, previously warmed to 37° C, and placed in the incubator. The glands are rinsed twice with warm isotonic salt solution and ground in the sterile tissue press of special design.

Then 1 cc of beef infusion broth P_H 7.4 is added with a pipette while the ground gland remains in the press and suspension accomplished by blowing back and forth.

Two cubic centimeters of sterile human ascitic fluid and 0.2 cc of the gland suspension are added to each of two tubes containing 15 cc of molten beef infusion dextrose agar P_H 7.4, cooled to 40° C. Each mixture is transferred to Noguchi tubes 14 by 200 mm and overlaid with 1 cc of sterile petrolatum. The tubes are incubated at 37° C and examined by transmitted light daily. All tubes are kept for six weeks before being reported as negative. To pick colonies from deep tubes the column of agar is transferred to a sterile Petri plate in the following manner. A sterile 8 mm glass tubing with a 1 mm thick wall is drawn out in the gas flame to make a capillary tube at least 25 cm long; the large end is inserted into a 50 cc rubber pressure bulb and the capillary warmed by passing through a flame. The capillary is pushed between the column of medium and the wall of the tube until the butt is reached. Pressure is applied to the bulb while the butt end of the tube is held over the flame. As soon as the column is dislodged, more air is forced into the butt and the column will slip easily by gravity into the Petri plate. The colonies are picked under a dissecting microscope and transferred to tubes of beef infusion blood broth, which are incubated anaerobically.

Parallel cultures from the gland are prepared by adding 0.2 cc of the suspension to rabbit blood agar plates, beef infusion dextrose blood broth and ascitic fluid dextrose agar slants.

Vaccines of these organisms consist of a forty-eight hour beef infusion broth culture heated to 60° C for one hour. After beating a culture is made from the vaccine to make certain that it no longer contains viable organisms.

LEUCOCYTES The Polymorphonuclear Count in the Newborn Sanford H. N. Am J Dis Child 38 547, 1929

From a study of 100 infants the author concluded that short exposures to the ultra violet light tend to increase the number of young or single lobed nucleated cells of the polymorphonuclears. The older or multilobed forms are decreased. There is a rapid return to normal.

BLOOD COUNT Relationship of Jaundice and Weight To Blood Values In The Newborn Infant Mitchell J. McK. Am J Dis Child 38 518, 1929

The erythrocyte count and hemoglobin content of the blood of 69 infants on the first, third, seventh, and tenth days of life are reported.

The percentage by volume of the cells in the blood of 50 newborn infants is also reported. The cell volume is considerably greater than the average value for adults. The volume index is also high.

Infants with jaundice do not show lower average erythrocyte and hemoglobin values than those without jaundice.

Practically all infants show a loss of erythrocytes and hemoglobin between birth and the tenth day of life. The average loss of infants with marked jaundice is only slightly greater than that of infants with no jaundice.

Relative changes in weight exert an effect on the loss of erythrocytes when the infants compared show the same degree of jaundice.

Infants with marked jaundice show a much smaller gain over their weight at birth on the tenth day of life than infants without jaundice. This exerts a leveling factor when one compares the loss of erythrocytes in the two groups.

Dehydration when of a degree sufficient to be plainly manifest clinically and to cause fever, exerts a marked influence on the erythrocyte count.

Changes in percentage by volume in the plasma may be sufficient to prevent a closer correlation between the intensity of jaundice and the loss in erythrocytes and hemoglobin than shown in this series.

HEMOGLOBIN Estimation of by Cell Concentration, Felsen, Jos Arch Path 8 484, 1929

1 Shake a few grains of dry, powdered sodium citrate into a clean dry capillary glass tube having an inside diameter of 4 mm and a length of 10 cm

2 Holding the tube in the horizontal position or with the distal end slightly depressed, apply the proximal end to a bleeding wound. A rapid, deep, incised wound of the finger made with a sharp lancet shaped needle will fill at least three such tubes with little or no pressure on the finger

3 Fill the tube about two thirds full and mix the blood with citrate by alternately elevating and depressing one end

4 Allow the blood to gravitate to one end, seal that end with a plug of paraffin and place a broad rubber band snugly around the length of the tube, thus sealing both ends. It is well to file the cut edges to avoid injury to the fingers or tearing of the rubber band

5 Centrifuge at high speed (plugged end down) for a sufficient length of time to secure the maximal separation of cells and plasma. This is determined as the period of time after which repeated centrifugation no longer diminishes the length of the cell column. Once established, the same button on the rheostat and the same period of time may be used with every specimen

6 File mark the capillary tube (a) at the junction of the paraffin plug with the cell column and (b) at the junction of the cell column with the plasma column. Break off at both points, thus isolating the cells

7 By means of a Sahli pipette, graduated at 10 and 20 mm, the tip of which is applied directly to either of the open ends of the cell column, draw up the cells to the mark "10" (10 cmm). Add this to the graduated Sahli tube containing tenth normal hydrochloric acid to the mark 10. Wash the pipette thoroughly by sucking up and expelling some of the same fluid. Shake the mixture well, and then allow it to stand at least one minute or until the maximal change of color has been effected. Dilute with water to match the standard. When the Dare instrument is used, aspirate the cells from the capillary tube by means of a Sahli pipette, as before, to the mark "10". Then continue aspirating physiologic sodium chloride solution to the mark "20". Eject the resulting mixture (10 cmm of cells and 10 cmm of saline solution) on to a hanging drop slide or a small watch glass. Mix thoroughly by alternating aspirating and expelling the mixture from the pipette four or five times. Having secured a uniform mixture, take it up in the Sahli pipette and fill the Dare automatic pipette, the 20 cmm of fluid will completely fill the latter. Compare in the colorimeter

The author concludes that when the cell plasma ratio $\frac{c}{p}$ approximates 1 (i.e., when the cell column and the plasma column are approximately equal), the estimation of the percentage of hemoglobin by the cell concentration method yields a value most nearly approaching that obtained by the old method

When the cell plasma ratio is low (i.e., when the plasma content of the blood is relatively high), the new method gives a considerably higher percentage of hemoglobin than the old. This is least marked when the red cells are impoverished in hemoglobin while the red cell count is high

In primary anemias, the cell plasma ratio being low, the percentage of hemoglobin is found to be much higher than with the old method

In chlorosis, the cell plasma ratio being high, the percentage of hemoglobin is found to be the same or lower than with the old method, by reason of the low hemoglobin content of individual cells

In polycythemia vera, the cell plasma ratio being high, the percentage of hemoglobin is found to be lower than with the old method

In secondary anemias associated with acute hemorrhage, the cell plasma ratio being low, the percentage of hemoglobin is found to be either normal or low. This is due to the fact that cells are lost, rather than hemoglobin, the percentage of the latter being frequently normal as estimated by the concentration method. The difference between the old method

and the new is due to the fact that the latter eliminates one variable factor the diluent plasma. The importance of this observation is evident in repeated examinations of a patient with bleeding gastric or duodenal ulcer. Watching the cell plasma ratio will be found a much more reliable index than estimation of the percentage of hemoglobin and the number of erythrocytes.

In secondary anemias associated with dehydration (cachexia, inanition), the cell plasma ratio being high, the percentage of hemoglobin will be found lower by the new method because the cells have already been concentrated *in vivo*.

These observations suggest a new concept of anemias. Anemias may be divided into

1 *Cytinemia*. This classification embraces the majority of the types of anemias and is due to a diminution in the number of the red blood cells. It includes secondary and primary (pernicious) anemias. The number of erythrocytes is low, the percentage of hemoglobin is normal or almost so, the color index tends to be high and the cell plasma ratio is low (less than 1).

2 *Hemoglobinemia*. This classification includes some secondary anemias and chlorosis. Here the percentage of hemoglobin is low, the number of erythrocytes is normal or increased, the color index is 1 or less and the cell plasma ratio is 1.

It is interesting to note that in *polycythemia vera* the percentage of hemoglobin is normal, the number of erythrocytes is greatly increased, the color index is 1 or less and the cell plasma ratio is high (more than 1).

Some secondary anemias and aplastic anemia fall in an intermediate group between 1 and 2. The foregoing simple classification of anemias is based on the determination of whether the cells or the hemoglobin are primarily involved. The use of the cell plasma ratio and the estimation of the hemoglobin content by the cell concentration method appear to be satisfactorily adapted for such a procedure.

ANEROBES Simple Method for Isolation of Wenzel T Arch Path 8 487 1929

For the culture of blood 2 cc of the extracted specimen is added to a tube containing about 15 cc of melted agar and the mixture poured into a Petri dish. After inoculation the plate is chilled in the ice box until the agar is firm. Sterile melted petrolatum is then poured over the surface of the plate to give a layer about 1 cm in depth. For the culture of surgical swabs, pus and other material the same procedure is used with the exception that plates are prepared with several different dilutions of the test material to insure obtaining discrete colonies.

The plates are incubated in the usual way.

No difficulty is experienced in the detection of colonies on positive cultures. When it is desired to pick the colonies for transplanting or for staining the plate is chilled and the hardened layer of petrolatum is easily lifted away with a sterile wooden tongue depressor. If the plate contains gas formers it is best to make transplants before the production of gas causes disruption of the culture medium and bubbling of the petrolatum layer.

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building,
Richmond, Va

*Vaccination Against Tuberculosis by Means of B C G**

IN VIEW of the widespread interest and discussion concerning the work of Calmette and Guérin in the prophylaxis of tuberculosis by vaccination, this pamphlet will be read with interest by all who are at all concerned with this problem

It represents the findings of three commissions, Bacteriologic, Clinical, and Veterinary, and presents concisely a rather comprehensive survey of this question

The Bacteriologic Committee, with one exception are agreed that B C G is a harmless vaccine and does not give rise to progressive tuberculosis

Prof Nobel, however, believes that under exceptional conditions B C G was capable of inducing fatal tuberculosis in laboratory animals

The following studies were deemed desirable

1 Methods to be adopted in order to maintain unaltered the fixed properties recognized as characteristic of B C G

2 Methods to be adopted for the study of the influence on B C G of passages through animals

3 Methods to be adopted for immunizing experiments, determination of doses of B C G and virulent bacilli to be used in them, adoption of strains of known virulence for the virulent inoculations

4 Methods to be adopted in the studies on the variability and dissociability of B C G

5 Methods to be adopted for the comparative study of the histologic changes produced by inoculation of B C G and of virulent tuberculosis bacilli

6 Necessity of entrusting the preparation of B C G (culture make and distribution of vaccinal emulsions) to institutes of recognized scientific standing

The Clinical Commission from the documentary evidence laid before it concluded that

1 B C G, when administered per os to infants within ten days after birth, or hypodermically to older children and adults, was incapable of producing virulent tuberculosis lesions

2 As regards the preimmunizing properties of B C G against tuberculosis, a certain degree of immunity was induced by this vaccination

Further research work upon vaccinated children, continuing for a more lengthy period and carried out in a uniform manner—and, in particular, a fuller knowledge of tuberculosis morbidity and mortality among individuals of varying age and environment—are necessary before the Commission is able to pass a final judgment on the value of antituberculosis vaccination with B C G

The conclusions of the Veterinary Commission were that

1 The mass of experimental data published and the unanimous opinion of practitioners who have used B C G upon bovidae indicate that vaccination performed according to the technique advocated by Calmette and Guérin upon animals of this species is entirely innocuous

*Report of The Technical Conference for The Study of Vaccination Against Tuberculosis
B Means of B C G League of Nations Health Organization 1928 Paper 147 pages World
Peace Foundation Boston

NOTE In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto

2 The same experimental data and the observations made of the practical use of B C G upon bovidae show conclusively that this strain of bacilli possesses preimmunizing properties against both experimental and natural tuberculous infections

These acknowledged preimmunizing properties provide justification and encouragement for the extension of the experimental use of B C G in the prophylaxis of bovine tuberculosis

The use of B C G should be continued in the various countries in accordance with the international experimental protocol which is attached under the supervision of official veterinary services and competent bacteriologic and pathologic authorities and in close touch with the Commission set up for the study of this problem by the Health Organization of the League of Nations

Wherever possible the trials should be carried out strictly in accordance with the experimental rules in other cases trials may be made more in conformity with the ordinary conditions of cattle rearing under continuous official supervision

The methods and plans for the further studies deemed necessary are set forth at length in the body of the report

There is but one criticism These reports, while of necessity temporary and to be supplemented by further data before final conclusions can be reached are nevertheless of great value as sources of reference and as such should be available in somewhat more durable form

*Outline of Preventive Medicine**

"**P**REVENTION of disease" says Dana in the Foreword of this book "involves at least two lines of action, one preventing the contraction of certain diseases the other preventing the development of serious symptoms of the disease contracted"

Thus "prevention of disease often means the prevention of the disturbing symptomatic effects of a fundamental specific disease invasion"

And again The usual attitude of the general practitioner toward preventive medicine is of course commendatory but not exactly enthusiastic

The reviewer would suggest that to no small extent this comparative indifference may be due to some small lack of clear understanding of the situation and its ramifications It might be taken as a somewhat striking commentary upon medical practice in general that, following the propaganda concerning the periodic health examination, it should have been felt necessary to demonstrate by moving pictures, etc, how to make a thorough physical examination

This fact, it seems is deserving of some thought Either such demonstrations were inculcating to the rank and file or the rank and file are due to consider whether they are competently practicing what they preach

This book it seems to the reviewer fills a needed want Simply written yet authoritative its perusal should arouse thought and give impetus to the necessary study required to practice preventive medicine in the fullest sense It may be read with profit by physician and layman alike

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him was the esteem of other great men and the love of his students. His family life was exemplary and he inculcated in his sons high ideals. It was his happy lot to live to see his work appreciated and his sons carry on successfully.

The World War brought three colonelcies, a majority, a captaincy, and a lieutenantcy to his family but it also caused the one shadow of his life when it took from him his first born son. The sorrow of this irreparable loss shattered the indomitable spirit of the man, he aged in spite of his will to carry on. He gradually withdrew from the more intense activities of life.

In 1921 at the age of seventy he resigned from his duties in the Medical School in the University of Michigan. It was a sad parting from his Alma Mater, the great institution that he had builded. He still had much to do in his chairmanship of the National Research Council, in launching *Hygiea*, in putting this, his own JOURNAL OF LABORATORY AND CLINICAL MEDICINE into order, and in writing the important story of his life, *A Doctor's Memories*. In his usual interesting charming way he peened the scenes that had passed by him in his long active journey through life. The panorama of American medicine and the outstanding men of his generation have been accurately and faithfully described by him as he saw them with his own quick analytical senses in close personal contacts.

Doctor Vaughan aroused in his students sentiments that are too hallowed to entrust to inadequate words. It would be a Herculean task to recount all of the deeds and accomplishments, the works and honors of a life that has been so extraordinarily full. A decision as to which of his attainments may be commented upon as his greatest depends upon what contact his biographer has had with him. He is outstanding in many fields. To his students, however, and to the great mass of medical men he was primarily a teacher and a preeminent pioneer in medical education. He has brought his stimulating influence to bear upon his colleagues as well as upon each individual of the mass of undergraduates who passed through his classroom or under his tutelage through the thirty odd years that he served successfully as professor of physiological chemistry and hygiene and dean of the Medical School of the University of Michigan. This institution is a monument to his genius, for here, in a small town he built a great medical school and maintained it in the first rank throughout his years as its dean. It still shines resplendent in glory of its past. He possessed an uncanny ability to choose his faculty members wisely and to organize and administer the school. Frequently men whom he had developed were taken from him by the heavily endowed institutions in the East to one of which he had to relinquish no less than seven of his men but he was always able to rebuild satisfactorily. A splendid nucleus of home talent he was finally able to hold against all inducements.

In the eyes of his students from the first day of classwork, Doctor Vaughan was the quintessence of medical scientists. He somehow inspired a deep sense of reverence and had a tendency to inspire hero worship, although he himself was never given to such. His was a very critical attitude of mind, but a sense of humor was its saving grace. He was sharply critical of himself as well as of others. He usually held himself in reserve, and successfully

mastered his inner thoughts. He insisted upon the three I's, industry, intelligence, and integrity. He lived up to the demands that he made of others. Deep sincerity of purpose and action were other marks of his greatness.

He traveled the highways of life with the great men of his age and has been able to count many of them among his intimate friends. Respect of the great stimulated him, but he lost not the common touch. His contacts with his students meant most to him. He always divided his classes into the sheep and the goats, but a sincere fatherly interest was with the individual even though he were among the goats. His outward show was not so fatherly as that of others, but in the storm and stress periods the students soon learned of the sincerity of his friendship. His was a personal contact with his "boys," each of whom he called by his Christian name. He always remembered some anecdote concerning each one, which he would relate at an opportune moment.

Friendship was to him sacred. He never grew blasé with the honors that were heaped upon him. He attributed whatever success he had attained to the loyalty of his friends. Inwardly he was a most modest soul. His outward bearing was never that of a feigned modesty, in fact, it was always such as to mask his feelings completely. Only the few, to whom years of association had given glimpses beyond the front, were able to realize his sensitive self-critical attitude. He was an aristocrat in the true sense of the word.

A great privilege and honor it has been indeed to know such a spirit and to have come under such an influence as his, to have been advised and guided by his genius. He has made a lasting impression upon the medical men of his generation. His job is done and well done.

The founding of this JOURNAL OF LABORATORY AND CLINICAL MEDICINE was simply an outgrowth of his pioneer work in which his vision and foresight perceived the importance of the laboratory in clinical medicine. The greatness of this vision has been justified. Such is the expression of our sentiments, however futile and inadequate our words may be.

—George Hermann

VICTOR C VAUGHAN

AN APPRECIATION

THE artist can neither paint the sunset nor gild the rose quite so well as he would like. The builder never creates with steel and stone quite so perfectly as he dreams. The writer never makes the children of his fancy so real as he desires. The worshiper never gives to his hero the halo that he believes the hero deserves. For these reasons, I am quite unable to pay the homage to Doctor Vaughan that my heart yearns to pay.

Doctor Vaughan was born in Mount Airy, Missouri, seventy eight years ago, and spent his boyhood not far from where another great Missourian Samuel W. Clemens, Mark Twain, passed his youth. The lives of both these men have proved to be a blessing and a benediction to mankind: the one for the undying service rendered his fellow men in the fight against disease, the other for the joy brought to countless millions through the written word. No crusader ever fought with a greater zeal than did Victor Vaughan in the practice and in the teaching of medicine. No one better understood the problems of the general practitioner than he. No one worked harder than he to help the general practitioner to solve them. Unlike many of his great contemporaries—Osler, Lister, Welch, Pasteur, Koch, Halsted—Doctor Vaughan's labors were not confined so much to hospitals as to the outside cases. In the homes to which he was called in counsel by the family physicians he did his most effective work. These contacts burned into his soul the knowledge that better trained general practitioners were needed and this need was the urge that drove him continuously on and on to build better and equip more scientifically until he made the Medical Department of the University of Michigan of which he was dean for thirty years one of the great medical schools of America.

Like most empire builders, Doctor Vaughan was blessed with a vivid imagination. He saw far into the future then with a stout heart and willing hands he labored long and earnestly to realize his dreams. Never shall I forget an evening spent with him in the library of his home at Ann Arbor listening to the story of when and how he first conceived the idea that the common house fly was a carrier of the deadly typhoid fever germ. "During the Spanish American War," said he, "I was sitting in the officers' quarters of the training camp in Chelamauga. Typhoid fever was raging. I had just come from the hospital where hundreds of patients with typhoid fever were under treatment. I had seen the latrines into which had been dumped the sewage from the hospitals. These latrines literally swarmed with flies. Why could not these flies be the same ones that I saw walling on the edges of drinking cups, cooking utensils, and even on food in the officers' quarters? Turning to a fellow medical officer I said 'Doctor these flies may be carrying on their feet enough typhoid germs to kill us all.' " Subsequent investigation has proved the correctness of this theory.

One who was privileged to sit at his feet can well understand how he could draw medical students to him and inspire them. His earnestness, his integrity, his zeal for service were contagious. His smile quickened the pulse, buoyed the spirit, renewed the courage, and brought greater hope to all who came in contact with him. It is not surprising that the graduates of the University of Michigan have been pathfinders in medicine in many lands, in many climes. They are carrying on as the old master bade them and they will continue to carry on in his name while there is one alive to revere his memory.

Preventive medicine was the most alluring field to him. "To keep people from getting sick is the goal of the true physician," said he. It was his great ambition to see a county hospital in every county in every state, and in these hospitals a well-trained competent staff willing and ready to serve. One of the most inspiring articles he ever wrote, "The Doctor's Dream," made vivid mention of these things. Doctor Vaughan abhorred medical politics. The Star Chamber sessions at state or national medical meetings for the purpose of furthering some ambitious doctor's dream for power had his profound contempt. Almost every gift that organized medicine could bestow on one was given him, but they came unsought.

To him more than to any other American physician credit is due for the place the medical laboratory now occupies in scientific medicine. In the October, 1915 issue of THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE he said editorially, "The man who attempts to practice medicine without laboratory aid, belongs to a past generation and fails to do justice to his patients or credit to himself."

He lived to see well-equipped, medical laboratories a part of every correctly managed hospital, group clinic, and private office. He lived to see more accurate diagnoses made and better medical service rendered to millions of people because of his teaching in this field of medicine. Much is being said and written today in scientific medical circles about allergy. An understanding of this phenomenon has taken much of the mystery away from certain diseases. Years ago Doctor Vaughan with prophetic vision predicted this. His work on protein poisons was the forerunner of present-day understanding of allergic reactions. Here, again, he lived to see his dreams come true. He, more than any other authority, counseled against the indiscriminate use of

dawned. This quality made him the great epidemiologist that he was, probably the greatest of his day. In peace or war, when an epidemic raised the black flag of death and sought to strike men down, his was the mailed fist that came to the rescue.

No more kindly, congenial spirit ever blessed the dwelling place of man than Doctor Vaughan. He was intensely human, a good companion, wise counselor, loyal friend and willing at all times to accept the fortunes of combat. Neither victory nor defeat swerved him from the even tenor of his way.

Easily the outstanding toxicologist and medical jurisprudence expert of his time, his great talents were never for sale to the strong to be used against the weak. He resented the technicalities so often resorted to in the courts for the purpose of defeating justice. Thus resentment caused him to retire voluntarily from this field after a noted murder trial in Kansas City in which he felt that justice had sadly miscarried. Great as Doctor Vaughan became in the field of science this made not the slightest difference in his relationship with his fellow men. Nothing but gentleness, kindness, brotherly love, and quiet marked his hospitality which was so much in evidence in his Ann Arbor home. Here he met the great and the small in medicine, and all were treated alike. The renowned professor, the humble medical student, the struggling country doctor, or even the wayfarer in the commercial desert whose wanderings brought him to Ann Arbor for a conference with Doctor Vaughan—all found a cordial welcome, and all left inspired and impressed.

Death as well as birth is a natural course. Short indeed is the race from the cradle to the grave. The eye grows dim, the heart is saddened over the passing of so great a man, but with this grief there comes a gladness in the realization that one such as he has lived to be a husband, a father, a teacher, and a friend.

—C V Mosby

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News and Notes

Dr J J Moore, Chicago, Illinois, presented a paper entitled "The Relation of the Record Librarian to the Clinical Laboratory" before the Convention of the Association of Record Librarians of North America, October 14 to 18, 1929, at Chicago, Illinois

The Official Representatives of the American Society of Clinical Pathologists to the Nineteenth Clinical Congress of the American College of Surgeons at Chicago, October 14 to 18, 1929 were as follows Dr Oliver W Lohr, Saginaw, Michigan, Dr Frank W Hartman, Detroit, Michigan, Dr William Thallner, Chicago, Dr H C Swaney, Chicago, Dr J J Moore, Chicago

Dr Frank W Hartman, Detroit, Michigan, presented "What Constitutes an Efficient Clinical Laboratory Service for a Hospital?" on the program of the Twelfth Annual Hospital Standardization Conference held in Chicago, October 14, 15, 16, and 17, 1929 Dr Oliver W Lohr, Saginaw, Michigan, opened the discussion of this paper

Dr Charles R Drake, Minneapolis, Minnesota, and Dr Kano Ikeda, St Paul, represented the Society at the Fifty eighth Annual Convention of the American Public Health Association in Minneapolis, September 30 to October 5, 1929

The Board of Registry of Technicians of the American Society of Clinical Pathologists has begun issuing certificates to those technicians successfully meeting the requirements These diplomas are being sent out as quickly as the investigation of the applicant is completed The Placement Bureau has been operating very successfully and several technicians have been placed in desirable positions You are invited to take advantage of the facilities offered by this department of the Registry

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RECENT DEVELOPMENTS IN TULAREMIA (FRANCIS DISEASE)*

WITH A REPORT OF ELEVEN ADDITIONAL CASES

BY WALTER M. SIMPSON, M.S., M.D., F.A.C.P., DAYTON, OHIO

TULAREMIA has abruptly become a common and widely recognized disease of man. Up to 1924 but 15 cases of tularemia in human beings had been reported. During the past five years, over 800 proved cases have been recognized in the United States. Cases have now been reported from every state in the Union, except the New England States, Delaware and Washington. The incidence ends abruptly at the Canadian and Mexican borders, despite the large number of cases discovered in border states.

Ohio ranks first among the states, with 92 recorded cases. Montana ranks second with 66 cases. Forty-five cases have been found in Washington, D. C. It is not without significance that the largest number of cases have been found in those states in which certain individuals have been actively engaged in investigating the disease. The obvious inference is that many more cases would be unearthed in other localities if special effort were made to find them.

For many years it appeared that tularemia was restricted to the United States. Recent reports indicate that it is probably a world-wide disease. Francis and Moore¹ demonstrated that "Ohira's disease" in Japan is identical with tularemia. Three laboratorians in London, England, developed the disease after performing autopsies on laboratory animals inoculated with cultures of *Bacterium tularense* sent by Francis at their request.

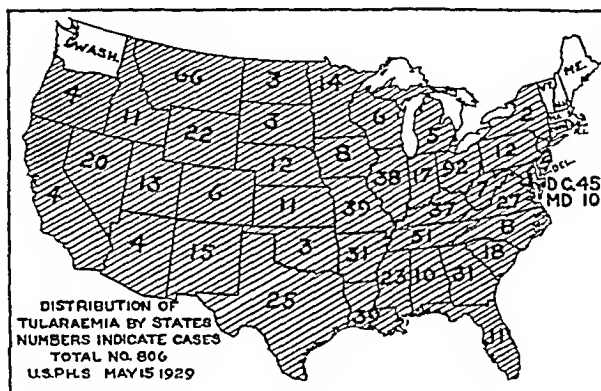
Four recent contributions by Russian workers, published during 1928, indicate that tularemia is probably widespread in the Union of Socialist

*From the Diagnostic Laboratories of the Miami Valley Hospital, Dayton, Ohio.
Read in part, before the Eighth Annual Convention of the American Society of Clinical Pathologists, Portland, Oregon, July 5, 6, and 8, 1929.

This paper received the Ward Burdick Research Award for the year 1929.

Soviet Republics Nikanorov³ tells of three extensive outbreaks of a "tularemia-like" disease. The first occurred in the province of Astrakhan, involving about 150 persons. The second large group of cases (over 100) was encountered in the province of Uraisk. Simultaneously, a tremendous number of cases (over 800) was discovered in the province of Riazan, on the Oka River.

All of the Russian cases resulted from direct contact with the fur-bearing water rat or water vole of Europe (*Avicola amphibius*) which is hunted for its fur. The onset of the epidemics in nine villages along the Oka River was associated with a flood which drove the water rats ashore in great numbers. The furs of these animals have considerable commercial value and the inhabitants of that region seized upon the opportunity to obtain large numbers of the animals with relatively little effort. The water rats are ordinarily pierced with a harpoon, which enters the body of the rat very readily but is withdrawn with great difficulty. The hands of the hunters are always covered with blood during the process of releasing the harpoon and skinning the animals.



the necrotic lesions revealed a gram negative coccobacillus. Three guinea pigs died after inoculation with material from some of the lesions, showing the characteristic pathologic anatomy of tularemia. From these lesions an organism, regarded by the writers as identical with *Bacterium tularensis*, was recovered on coagulated egg yolk media. Guinea pigs and white mice injected with the second generation of organisms died in three to five days following inoculation, with the production of the same gross lesions of the spleen, liver and lymph nodes, and yielding the same growth on egg yolk media. The organisms thus recovered agglutinated in high titer in the serums of human convalescents.

These ardent investigators paid the inevitable toll for their discoveries. Four Russian physicians acquired the disease during the course of these investigations, thus bringing the total number of laboratory infections with tularemia to twenty-four.

Professor Zarhi of Sverdlovsk (Ekaterinburg) in the province of Perm, Russia, sent serum of a patient thought to have acquired tularemia, to McCoy, at the Hygienic Laboratory at Washington. McCoy found that it agglutinated *Bacterium tularensis*; he also isolated *Bacterium tularensis* from guinea pigs which had been inoculated with guinea pig spleen tissue received at the same time from Professor Zarhi.

New animal and insect hosts and transmitters of tularemia have been recently discovered. Dieter and Rhodes⁶ found that tularemia exists in nature among wild rats which were trapped in Los Angeles, California. Perry⁷ isolated *Bacterium tularensis* from meadow mice (*Microtus californicus aestuarius*) in Contra Costa County, California.

Parker and Dide⁸ found evidence of present or recent infection with *Bacterium tularensis* in eight sheep (*Ovis aries*) of a large Montana band, a considerable percentage of which were affected by a similar illness. Parker is of the opinion that many of the heavy losses among sheep in that region have been due to tularemia. The sheep were known to be infested with the wood tick (*Dermacentor andersoni* Stiles)—a frequent host and transmitter of tularemia. Parker and Francis succeeded in recovering *Bacterium tularensis* from the spleens of guinea pigs which had been inoculated with wood ticks found on the sick sheep; the organism was likewise recovered from sheep tissues.

Green, Wade and Dewey⁹ had demonstrated that the muskrat (*Ondatra zibethica*) is experimentally susceptible to tularemia, but it is only within the past few months that Schwartz,¹⁰ of Montana, has reported the development of tularemia in two Japanese section laborers who had cooperated in skinning a muskrat. Mease,¹¹ of Florida, has reported a case in a human being of tularemia resulting from the skinning of opossums (*Didelphis virginiana*).

A case in a human being of oculoglandular tularemia resulting from contact with a woodchuck (*Marmota flaviventris*, ground hog) is included in the series of cases reported in this communication. The only other reported case of tularemia in a human being as the result of direct contact with a naturally infected woodchuck was reported to Francis¹² by Dr. J. T. Powell, of Gravette, Arkansas. A fifty-eight year old man cut his finger while skinning a wood

chuck which he had killed. Death occurred twenty-three days after the injury. The patient's blood serum agglutinated *Bacterium tularensis* in all dilutions to 1:1280.

Even though McCoy,¹³ McCoy and Chapin,¹⁴ and Wherry¹⁵ found the domestic cat (*Felis catus*) to be unaffected by inoculations with *Bacterium tularensis*, Green, Wade and Hanson^{16, 17} have accumulated evidence which would seem to incriminate the cat as an occasional naturally infected carrier. Francis,¹⁸ attempted to infect thirteen cats with tularemia, two kittens died of the disease, the other animals did not acquire the disease. Francis places the cat among the mildly susceptible animals.

I¹⁹ found dogs to be naturally immune to the disease.

The repeated observations of laymen and scientific investigators of the simultaneous decimation of wild rabbits and game birds in certain localities led to the suspicion that tularemia may have been the common cause. This possibility was strengthened by the discovery, by Parker and Spencer,²⁰ that the common rabbit tick (*Hemaphysalis leporis-palustris*), an important transmitter of tularemia from rabbit to rabbit, is also found on game birds. Parker and Spencer have produced tularemia experimentally in the blue grouse of Montana. Green and Wade²¹ have found the ruffed grouse of Minnesota to be just as susceptible to experimental inoculation with *Bacterium tularensis* as are guinea pigs and rabbits.

Parker²² recovered *Bacterium tularensis* from guinea pigs which had been inoculated with the tissues of five quail (*Colinus virginianus*, bobwhite) which had died after the ingestion of food contaminated with tularemia-infected tissue. Parker concluded that quail may at least be considered potential agents of human infection with tularemia. A recent report of natural infection of quail with tularemia by Green and Wade²³ lends support to Parker's beliefs.

The deer fly (*Chrysops discalis*) and the wood tick (*Dermacentor andersoni* Stiles) are common transmitters of tularemia in the northwestern states. Another tick, thought to be *Dermacentor variabilis*, has been the responsible transmitting agent in 25 human cases of tularemia in Arkansas, Oklahoma, Texas, Louisiana, Tennessee and Minnesota. Parker, Brooks and Marsh²⁴ have recently recovered *Bacterium tularensis* from wood ticks of the species *Dermacentor occidentalis* Newman, collected in San Benito County, California. Even though new animal hosts and insect vectors are being constantly discovered, emphasizing the ever-widening dissemination of the disease, the wild rabbit is by all odds the most important reservoir of infection.

DAYTON EXPERIENCE WITH TULAREMIA

In June, 1928, I²⁵ reported 48 proved nonfatal cases of tularemia in the human being, and one rapidly fatal case, occurring in Dayton, Ohio. A detailed report of the clinical, pathologic, bacteriologic, and serologic findings in the most rapidly fatal case of tularemia on record (four days, seven hours), together with a description of the experimental production of the ulceroglandular, oculoglandular and glandular forms of the disease in laboratory animals, appeared somewhat later.²⁶

Bacterium tulareense was grown directly from human tissues on artificial culture media for the first time, a human serum modification of Francis' cystine glucose meat infusion peptone agar was employed

It was demonstrated that tularemia had been prevalent in Dayton for twenty years and that during that time seven deaths, in all probability due to tularemia, had occurred

In November, 1928, four additional Dayton cases were reported

One purpose of the present undertaking is to report 11 recently discovered cases, thus bringing the total of the cases investigated by me to 64, sixty of which originated in or near Dayton, Ohio This is by far the largest number of cases to be recorded from such a circumscribed area

The pertinent clinical and serologic findings in the newly discovered cases follow

CASE 1—Mr G S, a forty year old farmer skinned and eviscerated a young ground hog (woodchuck) which his dog had caught while on an expedition through the woods with his boys on May 15, 1928 During the process of eviscerating the ground hog Mr S's son while playing with the liver of the ground hog ruptured the gall bladder and some of the bile entered Mr S's right eye Four days later the eye became intensely swollen and infected On June 12, 1928, Dr J W Payne health commissioner of Lawrence County, Ohio examined the patient and found the eye very much swollen, conjunctiva red as fire, multiple small yellowish arcs on both palpebral conjunctivae considerable cellulitis with edema about the right eye and painful enlargements of the parotid and submaxillary lymph nodes The submaxillary lymph nodes finally suppurated and broke through the skin about August 1 The patient experienced repeated chills night sweats and hot flashes The bones of the head and upper extremities were extremely painful The patient was unable to resume his duties until October, 1928

Serum of Mr S submitted to George W McCoy of the Hygienic Laboratory at Washington D C, on January 14, 1929 was found to agglutinate *Bacterium tulareense* in all dilutions to 1:160 Similar results were obtained by me

CASE 2—Mrs L B patient of Dr E E Bohlender a thirty six year old housewife, cleaned three wild cottontail rabbits which had been killed by her husband in Marion County Indiana, on November 1, 1928 While dressing the rabbits she cut the left index finger with a knife Two days later she experienced a sharp chill followed by a rapid elevation of temperature (104° F) She went to a physician in Indianapolis who made a diagnosis of influenza On the second day of her illness she observed that the left axillary lymph nodes were enlarged and painful During the next two weeks she experienced repeated chills and sweats, accompanied by feverishness, headache, backache, muscular and joint pain During the first week of her illness she was moved to Dayton, where Dr Bohlender recognized the case at once as one of tularemia

Serum of Mrs B, collected on November 15, 1928 was submitted to the Hygienic Laboratory at Washington, D C, where it was found to agglutinate *Bacterium tulareense* in all dilutions to 1:320 with partial agglutination in a titer of 1:640

Mrs B remained bedfast for two weeks after which the fever gradually disappeared and she made an uneventful recovery The axillary lymph nodes did not suppurate but slowly regressed in size over a period of five months

CASE 3—Miss A G, patient of Dr H H Stafford aged thirty two dressed three wild cottontail rabbits which had been killed by friends in Preble County Ohio on November 15 1928 Miss G had scratched the skin over the proximal phalanx of the right thumb two days before this On November 17 she developed repeated chills with a fever of 104° F She thought that she 'had a bad case of grippe' She remained in bed for one week during which time she experienced repeated chills and sweats On the third day of her illness she noticed for the first time a small ulcer which had developed at the site of

the scratch on the right thumb. The same day she noticed a painful swelling of the right axillary lymph nodes. The axillary mass did not suppurate.

When examined by me on December 1, 1928, Miss G was afebrile, a tiny healing ulcer was observed on the right thumb, and the right axillary lymph nodes were walnut sized and of firm consistency, suppuration did not occur. Convalescence was uneventful.

Serum of Miss G, collected on December 1, 1928, was found by George W McCoy, of the Hygienic Laboratory, and me, to agglutinate *Bacterium tularensis* in all dilutions to 1:160, with partial agglutination at 1:320.

CASE 4—Mrs A J, aged sixty six, patient of Dr D C Casto of Parkersburg, West Virginia. Her son, a resident of Dayton, came to my office on November 25, 1928, with the information that he had just received a letter describing his mother's illness and that he was certain from the description that his mother was a victim of "rabbit fever." He recited the details of his mother's illness and they justified his suspicions. I gave him a tube and a mailing container and insisted that he mail me a blood specimen as soon as he reached Parkersburg.

Serum of Mrs J, collected on December 1, 1928, was found by George W McCoy, of the Hygienic Laboratory, and me, to agglutinate *Bacterium tularensis* in all dilutions to 1:1280. In the routine test for agglutination of *Bacterium abortus* it gave a positive reaction in all dilutions to 1:80.

Through the courtesy of Dr Casto and as a result of an interview with the patient, who later visited Dayton, the following history was obtained. Mrs J dressed some forty or fifty wild cottontail rabbits which had been shot by her husband on their farm, during the week preceding the development of her illness. On November 16, 1928, while dressing rabbits, she perforated the skin of the right index finger with a needle like fragment of rabbit bone. On November 20, she experienced repeated chills, high fever, profuse sweats, aching pains in the head and extremities, accompanied by marked prostration. The original diagnosis was influenza. The right axillary lymph nodes rapidly enlarged to orange size.

A small circumscribed ulcer developed at the site of the perforation of the skin of the right index finger. This was incised without improvement on November 30.

Mrs J remained bedfast for four weeks, during which time she experienced repeated chills and sweats and lost considerable weight.

On January 2, 1929, Dr Casto incised the suppurating mass in the right axilla and two or three ounces of thick exudate escaped. A sinus tract has persisted at the site of the incision up to the present time (June 10, 1929).

Mrs J was unable to perform her household duties for three months. When examined by me on May 2, 1929, she appeared to be in good health. She has regained all of the weight which was lost during the acute illness.

Serum of Mrs J, collected by me on May 6, 1929, was found to agglutinate *Bacterium tularensis* in the same dilution as before (1:1280), with cross agglutination of *Bacterium abortus* in a dilution of 1:20. It is interesting to observe that the tularemia titer remained the same over a period of four months.

CASE 5—Mr F C D, patient of Dr J T Mackie, a twenty five year old gasoline station operator, killed five wild cottontail rabbits in the woods near Dayton on November 15, 1928. While climbing a fence during the hunt, he cut the skin of the palmar surface of the proximal phalanx of the left middle finger on a wooden post. While dressing the rabbits at home that evening, he contaminated this fresh cut with rabbits' blood. On the evening of November 17, he experienced a feeling of soreness in the region of the left elbow. On November 18, he observed a walnut sized mass in the epitrochlear area, during his work on that day he suffered from chilliness, feverishness, and weakness. The next day the fever reached 104° F, he was forced to quit his work, the epitrochlear mass had reached lemon size by this time. He remained in bed for five days, during which time he experienced repeated chills and sweats, severe headache and backache. On November 21, he noticed for the first time a walnut sized mass in the left axilla. A small, sharply punched out ulcer was found at the site of the wound on the left middle finger on November 23. He returned to work on November 25, still feeling feverish and weak.

When examined by me on January 24, 1929, a small lemon sized fluctuating epitrochlear mass was found, as well as a firm, nonfluctuant walnut sized axillary mass. The primary lesion was almost completely healed. Serum collected at this time was found by George W. McCoy of the Hygienic Laboratory, and by me, to agglutinate *Bacterium tularensis* in all dilutions to 1:1280.

On January 31, 1929, partial excision of the epitrochlear mass was done. Approximately three ounces of thick yellowish exudate escaped. Microscopic examination of the tissue of the wall of the abscess showed multiple foci of caseous necrosis, surrounded by epithelioid and fibroblastic granulation tissue containing many giant cells of the Langhans type together with diffuse lymphocytic and polymorphonuclear infiltrations.

CASE 6—Mr. F. C., patient of Dr. A. W. McCally, a twenty-eight year old factory worker, hunted rabbits near Lenoir City, Tennessee, on December 26, 1928. While cleaning several rabbits on that evening he perforated the skin of the pad of the left thumb with



Fig. —Mr. F. C., Case 6. Acneiform papular eruption of posterior cervical region. Appeared ten days after onset of illness. Disappeared in two months.

a sharp fragment of rabbit bone. Four days later he experienced repeated chills of moderate intensity accompanied by periods of feverishness and sweating. The extent of the fever is not known. On December 31 the patient complained of painful masses in the left epitrochlear region and in the left axilla. On January 1 he noticed for the first time a small ulcer of the left thumb at the site of the perforating wound. A Tennessee physician assured him that he had influenza. On January 7, the primary lesion was incised, no exudate escaped.

Mr. C. felt very weak for several days but refused to take to his bed. Ten days after the onset of his symptoms an acneiform papular eruption appeared over the back of the neck, over the dorsum of the hands and over the flexor surface of both forearms.

Mr. C. came to Dayton in search of work on January 15, 1929. He consulted Dr. McCally on January 19, who immediately arrived at the proper diagnosis. When examined by me on January 21 the eruption was unusually distinct (see Fig. 2). Firm walnut sized masses were found in the left epitrochlear and axillary regions.

Serum of Mr C, collected on January 21, 1929, was found by McCoy and me to promptly agglutinate *Bacterium tularensis* in all dilutions to 1 1280

When examined two months later, the eruption had cleared except for a few small lesions on the neck. The epitrochlear and axillary lymph nodes were of cherry size, firm, and gave no evidence of suppuration.

CASE 7—Mr W R, patient of Dr O C Henderson, a forty five year old proprietor of a fish and poultry market, skinned and dressed several cottontail rabbits on November 15, 1924. The rabbits had been shipped to Dayton from St Louis, Missouri. While dressing the rabbits, Mr R scratched the middle finger of the left hand with a spicule of rabbit bone. On November 21, the left arm "felt hot and heavy," and he noticed a small egg sized mass in the left axilla. The next day he noticed a small pea sized papule on the left middle finger at the site of the scratch, the center of the papule sloughed out and left a small, deep, punched out ulcer. Hot flax seed poultices were applied to the axillary mass for three weeks, at the end of which time spontaneous evacuation of about two ounces of thick yellowish exudate occurred. Drainage persisted for over one month.

Because of the necessity of his being at his market each day during his illness, Mr R did not take to his bed, but was forced to sit quietly most of the time because of feverishness, chilliness, and marked weakness. Mr R did not undertake full time work for three months. He knew at the time that he was a victim of "rabbit fever."

Serum of Mr W R, collected on May 16, 1929, four years and six months after the onset of illness, was found by McCoy and me to agglutinate *Bacterium tularensis* in all dilutions to 1 160, with partial agglutination at 1 320.

CASE 8—Mr F R, patient of Dr O C Henderson, brother of Mr W R (Case 7), a fifty two year old electrician, was employed by his brother during the fall of 1916 as a meat handler. Mr F R, while cleaning fish, the week before Thanksgiving, 1916, stuck a frozen fish fin under the nail of the right middle finger. He also perforated the skin of the tip of the fourth finger of the left hand in a similar manner on the same day. He did not actually dress rabbits on this day but did handle knives which other meat handlers had used in the cleaning of rabbits.

Two days after these injuries, both arms became swollen and painful with reddish purple streaks extending from the points of injury of both hands to the axillae. The axillary lymph nodes of both sides rapidly increased to lemon size. On this day, the fever reached 104°, being initiated with a severe chill. This was followed by severe chills and sweats for three weeks, during which time Mr R was bedfast and experienced several periods of delirium. The first physician who was called made a diagnosis of pneumonia. A consultant made a diagnosis of infection of the fingers with blood poisoning. Multiple nodules developed along the lymphatics of both arms. The axillary nodules and those of the arms suppurred and were surgically incised. Drainage continued for four weeks. At the end of the third month a suppurating nodule appeared in the right posterior axillary fold. This was incised and drained for three weeks. Mr R was unable to do any work for six months. During the acute illness he lost ten pounds. He has been perfectly well since the end of the long convalescence. He emphatically avers that he has never touched a rabbit since this experience.

Serum of Mr F R, collected on May 31, 1929, twelve years and six months after onset of illness, was found by McCoy and me to agglutinate *Bacterium tularensis* in all dilutions to 1 80—a titer which is in accord with that found in other long recovered cases.

The following three cases, occurring outside of Dayton, were brought to my attention as the result of serologic studies carried out at the request of the physicians indicated. The clinical details are furnished through the courtesy of these physicians.

CASE 9—Mr H H, patient of Dr John Thomas Bowen, Clearwater, Florida, a sixty nine year old retired business man, discovered a dead wild cottontail rabbit on the Bellevue Baltimore golf links on the morning of February 14, 1929. Influenced by the belief that the

possession of the left hind leg of a rabbit frequently improved one's luck, Mr H cut off the extremity. He observed that the animal was warm and flexible at the time of the dissection. During the amputation Mr H broke a bone in the rabbit's leg and perforated the skin of his left thumb with the sharp end. He squeezed the thumb, causing it to bleed freely, sucked the wound for a few moments and promptly dismissed the incident from his mind.

On the evening of February 16, some sixty hours after the episode described above, Mr H experienced chilliness, malaise, feverishness (102° F) and headache. Physical examination by Dr Bowen revealed an injected pharynx and a slightly enlarged spleen. The urinalysis showed a trace of albumin and occasional hyaline casts. A blood count showed 13,200 white blood cells.

On February 18 Mr H complained of painful swelling of the left thumb, and pain in the left axilla. Dr Bowen discovered that the axillary lymph nodes were swollen to lemon size. The left epitrochlear nodes were enlarged to walnut size. In view of the suggestive history, Dr Bowen suspected tularemia, while a surgical consultant insisted that the case was one of ordinary pyogenic infection. During the next week the temperature varied between 101° and 103° F with recurring chills.

Mrs H, a lay member of the board of trustees of the Miami Valley Hospital, of Dayton, who was familiar with the work on tularemia which had been carried out in this laboratory, was wintering at the same hotel as the H family. On February 20 Mrs S visited Mr H and expressed her conviction that he was suffering from tularemia.

On February 28 a macular and maculopapular eruption appeared over the face and upper anterior surface of the chest.

A long distance telephone conversation with me strengthened the belief that Mr H was suffering from tularemia. At my request blood specimens were submitted to the Hygienic Laboratory at Washington, D. C. and to this laboratory where the serum of Mr H, collected on March 4, 1929, was found to agglutinate *Bacterium tularensis* in all dilutions to 1:1280.

The patient's temperature reached the normal level for the first time on March 7. The left axillary and epitrochlear lymph nodes remained firm and tender, and showed no evidence of suppuration up to the time of the last examination on March 18, 1929.

CASE 10—Mr C, patient of Dr Franklin T. DuBois, Liberty, Indiana, a forty year old farmer killed several rabbits during the early part of December, 1916. A few days after dressing the rabbits a dime sized, indolent necrotic ulcer developed on his left thumb at the site of a deep fissure in the skin acquired while shucking corn some time before. The left axillary and epitrochlear lymph nodes became painfully enlarged at the same time. Mr C distinctly recalls chills, fever, sweats and backache. While he did not feel sick enough to remain in bed for any length of time he was incapacitated for work for four months. The axillary adenopathy gradually subsided. The epitrochlear mass suppurated and was drained surgically, attempts to recover an organism on ordinary media from the exudate which escaped on incision were unavailing.

Serum of Mr C submitted to me on February 26, 1929, twelve years and two months after the onset of illness was found to agglutinate *Bacterium tularensis* in all dilutions to 1:80. This finding was confirmed by McCoy, at the Hygienic Laboratory.

CASE 11—Mr B, patient of Dr Glen Nisley, Chillicothe, Ohio, a fifty year old meat handler, cut the palmar surface of the right index finger with a knife while dressing cotton tail rabbits in a market during November, 1927. A few days after the injury a deep ulcer developed at the site of the injury to the finger. A painful right axillary mass soon reached the size of a large orange. The development of the ulcer and adenopathy was simultaneous with the occurrence of recurring chills, high fever (103° F), sweats, severe headache, backache and joint pains. The axillary mass supplicated and was surgically drained, a sinus tract persisted for several weeks. Mr B was unable to perform any work for eight weeks.

Serum of Mr P, submitted to me on January 14, 1929, was found to give positive agglutination of *Bacterium tularensis* in all dilutions to 1:160. Specimens submitted to Dr R E Bower, Health Commissioner at Chillicothe, and to George McCoy, at the Hygienic Laboratory, gave identical results.

SUMMARY AND CONCLUSIONS

- 1 Recent advances in our knowledge of tularemia indicate that it is a world-wide disease
- 2 Over 1000 cases have recently been discovered in the U S S R (Russia)
- 3 Over 800 cases have been reported during the past five years from forty states and the District of Columbia in this country
- 4 Sixty-four proved cases have been investigated by me, sixty of which have occurred in one community—Dayton, Ohio
- 5 While the wild rabbit continues to be the great reservoir of infection, many new animal hosts (wild rats and mice, sheep, muskrats, opossums, woodchucks, cats and game birds), and new insect vectors have been discovered during the past few years, thus pointing to the ever-widening dissemination of the disease among lower animal life and to new sources of infection for human beings

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DISCUSSION

Dr J C Geiger—The number of reported cases of tularemia in the medical literature have been considerably augmented by an interesting survey of Nevada by Doctor Meyer and myself of the George Williams Hooper Foundation for Medical Research of the University of California Medical School Laboratory proof of 50 cases and epidemiologic evidence of 234 others with no deaths were submitted. All were of the gland and ulcer type with one exception, which was classified as typhoidal. The cases were stated to have been first observed in Nevada near Battle Mountain in 1912. This disease, having its bacterial origin discovered on the Pacific Coast and named accordingly, has been singularly absent or very few to be noted in the reports of these states. Perhaps, as this survey seems to indicate the disease prevails to an unusual extent at least in Nevada. Likewise there appears to be every type of known transmission rabbits, flies ticks, contact with other animals apparently healthy such as dogs coyotes, and sheep and such extraneous material as knife blades and barbed wire. Moreover, one proved case was attributed to bites or mosquito though the report admits this information was most vague. The question of immunity of the Indians in Nevada, who use rabbits as sources of meat to tularemia has been settled since these investigators submit laboratory proof of one case in a Pute and record of others in the Shoshones.

Dr W T Cummins—I enjoyed very much the presentation of this paper and the discussion of Dr Geiger. In the Southern Pacific General Hospital San Francisco in a period of thirty days, we had seven tularemia patients from Nevada. Dr Geiger rendered very valuable assistance along the Southern Pacific Lines in calling attention to the menace of tularemia. Our seven cases were apparently the result of three rabbit contacts three tick bites, and one fly bite. They made uneventful but protracted recoveries.

Dr C W Bonyne—We have just recently found a case of tularemia in a cattleman from Utah. The mode of infection was not definitely determined but it was felt that it came through an insect bite. This man had an intense preauricular and submaxillary adenitis. An interesting point which might be brought out in these cases is that the literature leads one to believe cross agglutination between *Bacterium tularensis* and *Brucella abortus* and *melitensis* is quite frequent. Francis states that this was true in 25 per cent of his cases, and in several the titer was practically identical with all three organisms.

Our case of tularemia shows no agglutination with *Brucella* and in our *abortus* work cross agglutination with *melitensis* is very rare. I do not believe cross agglutination between tularemia and *Brucella abortus* can be depended upon.

Dr Warren T Vaughan—I want to ask Dr Simpson in regard to the long drawn out cases whether he suspects that there may be a chronic form or whether there is a pos

sibility of a carrier state? Is there involvement to the central nervous system? Some of the sequelae which he has described suggest that there may have been a central nervous invasion

Dr Robert F E Stier—I can truthfully say that we in the Northwest have been on the lookout not only for undulant fever but for tularemia. We found one suggestive case. This patient was only in Spokane as a patient. His home was in British Columbia. The only thing we can say of it was that the histologic picture of the large mass in the axilla was suggestive of tularemia. I have been trying to get some blood from that patient ever since then but have been unable to. So far as Spokane is concerned I have been on the lookout for it. I have some of Dr Francis' antigen and also have sent some blood back to Dr Francis for checking. In three or four tests that were compared the results were all negative. It is possible that a diagnosis of typhoid is being made in the acute cases, so when the general practitioner does not recognize these conditions the laboratory man has nothing to go by.

Dr Frederic E Sondern—Might we ask Dr Simpson to say a few words relative to safe guarding of the laboratory workers?

Dr Walter M Simpson (closing)—It is true that only four cases of tularemia have been reported from California. This is of unusual interest in view of the fact that the disease was first discovered in the ground squirrels of Tulare County in that state. None of the cases of tularemia in human beings has been traced to contact with the ground squirrel. The ground squirrel tick is apparently a feeble transmitter of the infection.

As regards the cross agglutination of *Bacterium tularense* and the *Brucella*, Francis and Evans found that 37 of the sera from 100 cases of tularemia in the human being showed cross agglutination of *Brucella abortus* and *Brucella melitensis*. In only three instances were the antitularense and antiabortus melitensis agglutinins identical. Of the remaining 63 sera, many of which were of high antitularense titer, there was no cross agglutination.

The history of contact with infected rodents, flies, and ticks and the proportionately higher titer reached by antitularense agglutinins usually leaves no doubt as to the proper interpretation of the serologic findings. In the event of cross agglutination in those rare instances in which the agglutinin titer of all three organisms is the same or nearly the same, agglutinin absorption tests may be employed. As regards the serum agglutinins in tularemia, all investigators agree on these four points: first, that agglutinins do not develop until after the first week of illness, second, that agglutinins once acquired never disappear from the serum, making it possible to determine whether or not a person has had the disease at some time in the past, third, that subsequent exposure to the infection does not elevate the declining trend of the titer, fourth, that one attack confers a permanent immunity.

In answer to Doctor Vaughan's question, I would state that in the prolonged cases in which the patient has been incapacitated for many months, the acute illness lasted from two to three weeks. No one has found the organism in the blood after the twelfth day. The convalescence in these cases is characterized by extreme prostration. I do not believe that there is any actual central nervous system involvement, but that the nervousness, restlessness and insomnia are associated with the general debility. There is no evidence that the individual suffers from repeated recurrences of the disease.

It is hazardous to make a diagnosis of tularemia from tissue sections alone. Many tissue pathologists have confused the granulomatous lesions of tularemia with those of tuberculosis.

A RECENTLY ISOLATED BACILLUS OF THE HEMOPHILIC GROUP*

By F W HARTMAN, M D, AND EDNA JACKSON, M S DETROIT, MICHIGAN

THE recent epidemic of influenza again sharpens our interest in the etiologic factor or factors in this disease and also in Pfeiffer's bacillus which was described in 1892 and was considered the most probable organism concerned at least up until the 1918 1919 epidemic. One of the facts which demonstrates Pfeiffer's bacillus to be pathogenic for man is the production of meningitis with a 10 per cent mortality as shown in the 82 cases reviewed by Torrey.¹ Anderson and Schultz collected 94 cases occurring between the 1889 1890 epidemic and the 1918 1919 epidemic.

When a small gram negative coccobacillus, aerobic and hemophilic, was isolated from a ten year old child in the service of Dr J C Montgomery, a provisional report of Pfeiffer's bacillus was made while further cultural studies and animal work were done.

ISOLATION OF ORGANISM

The patient was a white male child ten years of age. A history of chicken pox shortly before the onset of the present illness two and one half weeks previously was given. The present illness began with weakness, drowsiness, and impaired strength in the right leg and foot. On admission the patient was drowsy and irritable and there was slight rigidity of the neck. Kernig's sign was positive and there was papilledema in both eyes. Temperature ranged from 101 to 104° F. Laboratory examinations showed, blood hemoglobin 11.3 gm per 100 cc erythrocytes 4,584,000 leucocytes 7,400, polymorphonuclears 74, small mononuclears 22 large mononuclears 4. Urine albumin +, sugar 0, hyaline and granular casts ++. Spinal fluid, slightly turbid distinct pellicle formed in twelve hours, leucocytes 90 per cm all lymphocytes, sugar 36 mg per 100 cc. Culture small gram negative coccobacillus. The organism obtained from the spinal fluid of this case could not be distinguished in morphology from *B influenzae*. Its cultural and morphologic characteristics are described below.

Morphology—The organism referred to in this article as culture 155, occurs as a small slender bacillus 1.2 microns in length, but more commonly as a small coccobacillus 0.6 to 0.8 microns in diameter. It is nonmotile capsules have not been demonstrated and spores are not formed. A few longer forms, 2 to 5 microns have been seen, but these are not common.

Staining Reactions—The organism is stained by the usual aniline dyes. It is gram negative. When stained with Giemsa's the center of the bacillus stains more deeply than the extremities.

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From the Laboratories of the Henry Ford Hospital

Cultivation—The organism is distinctly aerobic, and when first isolated could be grown only upon media enriched by blood or yeast extract

Best growth is obtained on rabbit blood agar. On this media a good growth occurs in from eighteen to twenty-four hours. The colonies are moist, translucent, mucoid in appearance but only slightly viscid in consistency. There is a marked tendency for the growth to become confluent, isolated colonies occurring only at the edges of the growth on an agar slant, or on plates where the inoculation was sparse. The isolated colonies are often irregular in outline, and may attain a diameter of 2 to 3 mm. Maximum growth occurs in forty-eight hours, after this the growth flattens down and the mucoid appearance becomes less marked. No hemolysis occurs.

Dextrose ascitic agar cultures generally produce a fair growth, although this is much less profuse and less constant than that on blood agar. The growth is moist and the colonies confluent.

Plain agar cultures were successful only after the organism had been carried on artificial media for three months. The growth on plain agar is very scant, being scarcely visible after two days. Yet fifteen successive transplants were carried out on plain agar. Smears made from plain agar generally show the small bacillus forms.

Dextrose hormone broth is evenly clouded and there is no pellicle formation. Smears made from this media show small coccobacilli more frequently than the slender bacillus form.

Fermentation Reactions—Sugar fermentation tests were made in tubes of Dunham's peptone broth to which were added 0.1 c.c. of yeast extract and 1 c.c. of 10 per cent solution of the sugars to be tested. Acid is produced in dextrose, levulose, galactose, mannite, saccharose and xylose. No acid is formed in maltose or lactose. Litmus milk is not changed.

Indol tests have usually been positive. Nitrates are reduced to nitrites. Gelatin is not liquefied. Tests for production of amylase as described by Rivers³ are negative.

Toxicity—Rabbits were inoculated intravenously with 5 and 10 c.c. of (1) saline suspensions of the organism killed by heating at 56° one hour, (2) seven-day broth cultures killed by heating at 56° one hour, and (3) seven-day broth cultures which had been filtered through a Berkefeld filter and tested for sterility. None of the rabbits showed any toxic effects.

PATHOGENICITY

In all 45 animals, 2 guinea pigs, 3 monkeys, 17 rabbits, and 23 dogs, were used. In these the organism was almost invariably fatal for all except the monkeys which resisted large doses intravenously, intratracheally and intracerebrally. It must be said however that the organism had lost much of its virulence for other animals before the monkeys could be obtained. The pathology produced is best studied with reference to the site of inoculation.

Of the two rabbits and four dogs inoculated intracerebrally, both rabbits and three dogs developed meningitis and the gram-negative bacillus was seen

in direct smears and recovered in cultures in pure form. The fourth dog was semicomatose for three days and then improved, but gradually lost weight and was sacrificed on the eighteenth day. Brain was negative on gross exami-



Fig 1—Lungs from dog one week after intratracheal injection of 10 c.c. of twenty-four hour broth culture of gram negative bacillus. Both lobes on the left and the lower lobe on the right are consolidated. Small abscess cavities stud the pleural surface. On section much greyish red old purulent material exudes from the surface.



Fig 2—Lungs from dog forty-eight hours after intravenous injection of 7 1/4 c.c. twenty-four hour broth culture of gram negative bacillus. The lobes are voluminous and firmly consolidated. On section the parenchyma is dark red in color having the general appearance of blood clot.

nation and culture. The lower lobe of the left lung was partially consolidated and a hemolytic streptococcus was recovered in pure culture. The gram negative bacillus was not recovered although the course was similar to that of other animals that survived the first few days.

The course in the meningitic animals varied from one to four days. All were drowsy or semicomatose and showed neck rigidity. The gross lesions were characterized by localized or general purulent and often hemorrhagic exudates over the cortex extending about the pons and medulla. Microscopically there was edema and swelling of the meninges and in most cases hemorrhage throughout. Of the cellular infiltration leucocytes and eosinophiles predominate with a good proportion of round and wandering cells. The brain substance was not involved but in some areas there is a marked perivascular infiltration.



Fig 3—Low power through wall of bronchus and parenchyma. The lining epithelium is denuded and the adjacent tissue necrotic taking a uniform red color as contrasted with the tissue further away which shows much emphysema and hemorrhage.

One rabbit and one guinea pig, each inoculated intraperitoneally, showed a diffuse peritonitis, in the pig the reaction was hemorrhagic, in the rabbit more purulent. The gram-negative bacillus was recovered in both instances in pure culture.

One guinea pig inoculated subcutaneously developed an abscess at the site of inoculation with the surrounding tissue infiltrated by blood.

The thirteen rabbits and five dogs inoculated intravenously (dosage ranged from 1 to 2.75 cc for rabbits and up to 10 cc for dogs) showed localization

principally in the joints, lungs, pleural, and peritoneal cavities. The rabbits were more prone to joint lesions while the dogs with one exception showed more marked lesions in the lungs. The spleen was enlarged and congested as were also the liver and kidneys but only exceptionally were abscesses or hemorrhages found in these organs. Diffuse hemorrhagic lesions including the intestine were seen twice.

The joint lesions were characterized by collections of grayish yellow or hemorrhagic exudate in the bursae and joint cavity. Erosion of joint surfaces was not observed.



Fig 4.—Low power lung parenchyma including two bronchioles after intratracheal inoculation. The lining of the bronchioles is denuded and the parenchyma is infiltrated with blood.

The peritoneal exudates were usually thick, viscid and stringy but in some cases were comparatively thin and only slightly turbid. On the other hand the pleural involvement was more often hemorrhagic both as regards the surface and the exudate. In a few instances both visceral and parietal pleura and the pericardium were thickened and hemorrhagic.

The lungs were similar in the animals inoculated intravenously and intratracheally as far as gross anatomy is concerned. The intratracheal dosage ranged up to 10 c c and was administered during morphine narcosis and by

means of a De Vilbiss sprayer. The lungs of animals succumbing in the first three days showed consolidation of one or more lobes with areas of consolidation in other lobes (Figs 1 and 2). Often all lobes were solid and hemorrhagic throughout. The visceral pleura was smeared with the hemorrhagic pleural exudate. The glands about the hilus were enlarged, soft and dark red on section. The trachea and larger bronchi showed slight congestion and never ulceration. The smaller bronchi were filled with thick, viscid, grayish-red exudate. The cut surface of the early lesions was dark red and firm but contained some air. In later lesions the cut surface was softer, grayish-red in color and oozed



Fig 5—Low power lung parenchyma through wall of bronchus the lining epithelium is denuded and the adjacent parenchyma is necrotic. Farther away are enlarged blood vessels and hemorrhagic emphysematous parenchyma is seen.

thick, viscid, grayish purulent material. Animals living two weeks or more often showed small abscesses throughout all lobes. These were filled with thick, grayish-red, viscid, purulent material and were not well walled off.

Microscopically there are no certain differential points to determine site of inoculation, but the lesions vary considerably according to age. The early lesions are characterized by exudation in the small bronchi and bronchioles and often desquamation or necrosis of the lining epithelium (Figs 3 and 4). The

alveoli adjacent may contain fluid or blood or both but the alveolar walls are thickened and infiltrated by round wandering cells and leucocytes. The blood vessels were engorged, and in many cases the walls of the smaller vessels are infiltrated with blood while there is a collar of blood immediately about the vessel. In lesions of three days' standing or more the alveoli are filled with leucocytes and still later definite abscess formation may occur with or without retention to the bronchi. Emphysema is a prominent feature of the earlier lesion with dilated and confluent alveoli scattered abundantly and diffusely through the lung (Fig 5).

Since the morphology and the growth requirements of culture 155 when first isolated suggested that the organism might belong to the group of influenza bacilli, three cultures of this organism and four strains of *B. influenzae* were compared in regard to some of their growth requirements. Three of the cultures of *B. influenzae* were obtained from Parke Davis & Company, one No. 211 from the Detroit Board of Health Laboratories. The latter had been recently isolated from the spinal fluid in a case of influenza meningitis.

The autoclaved blood was prepared by adding sterile rabbit's blood (5 per cent) to hormone broth. This was autoclaved for twenty minutes at 20 pounds, filtered, tubed and sterilized at 15 pounds for thirty minutes. To each one half of the tubes, 0.1 cc. of yeast extract was added. All cultures were kept ten days before being called negative. The results are shown in Table I.

TABLE I
COMPARISON OF GROWTH REQUIREMENTS OF CULTURE 155 AND FOUR CULTURES OF
B. INFLUENZAE

	155	156	157	<i>B. INFLUENZAE</i>			
				01765	01875	01291	211
Hormone broth	slight	slight	light	-	-	-	-
Hormone broth plus whole rabbit's blood	+	+	+	+	+	+	+
Hormone broth plus autoclaved blood	+	+	+	-	-	-	-
Hormone broth plus autoclaved blood plus yeast	+	+	+	+	+	+	+
Dextrose agar	slight	slight	slight	-	-	-	-
Plain agar	very slight	very slight	very slight	-	-	-	-

From these results it is evident that this organism is much less strict in its growth requirements than the true influenza bacillus since it will grow upon media containing only the thermostable factor in blood, or upon media enriched by aseptic fluid or yeast, even showing slight growth upon plain agar.

Since this organism bears some resemblance to bacilli of the *Pasteurella* group and since cases of human infection with members of this group have been reported (Mayer and Hopph⁴) a comparison was made with four strains of *Pasteurella cuniculicola*. Three of these strains were obtained from Parke Davis & Company one No. 131, from the American Type Culture Collection. These four strains agreed in fermenting maltose and not fermenting xylose.

thus differing from the fermentation reactions of the organism discussed here. None of these four cultures showed the translucent growth on blood agar that has been so characteristic in the cultures of this organism, yet that characteristic might be possessed by some strains. Moreover the bipolar staining characteristic of the *Pasteurella* group has never been observed in the study of this organism either in smears from cultures or in smears from tissue of inoculated animals.

TABLE II

COMPARISON OF CULTURAL REACTIONS OF CULTURE 155, AND TWO STRAINS OF *PASTEURELLA CANICULICIDA*

CULTURE	155	01315	131
Dextrose	A	A	A
Levulose	A	A	A
Galactose	A	A	A
Mannite	A	A	A
Maltose	-	A	A
Lactose	-	-	-
Saccharose	A	A	A
Xylose	A	-	-
Gel (h _q)	-	-	-
Indol	±	+	+
Nitrates	+	+	+

From time to time various gram-negative bacilli have been reported which bear a marked resemblance to Pfeiffer's bacillus in morphology and manner of growth, but which cannot be classified as belonging to that group because of differing growth requirements. Among these there might be mentioned *Hemophilus para influenzae*,⁵ described by Rivers, *Hemophilus canis*, originally isolated by Friedberger,⁶ and *Bacillus meningitidis cerebrospinalis septicemiac* isolated by Cohen⁷ from three cases of meningitis.

Hemophilus canis resembles the organism which we have described here in its fermentation reactions, in nitrate reduction, but differs from it in its opaque growth on blood agar, and in its lack of pathogenicity for rabbits and guinea pigs. Also it is apparently more strict in its growth requirements.

Cohen's organism has much in common with culture 155, in that it is essentially hemophilic yet can be grown upon dextrose ascitic agar, it shows a translucent confluent growth upon blood agar, it occurs as small bacillus similar to Pfeiffer's, also as small coccobacillus, especially in broth, it stains more deeply at the center than at the extremities, and in animals it produces a true septicemia.

From these studies it would seem that this organism isolated from a case of meningitis must belong to a group of organisms essentially hemophilic, yet not as strict in their growth requirements as either the true influenza bacillus or the para influenza bacillus described by Rivers. Therefore, if this organism is isolated in additional cases and continued observation confirms it as a distinct species, the designation *Hemophilus para influenzae B* is proposed.

SUMMARY

1 An essentially hemophilic organism isolated from a case of a fatal meningitis is described.

2 Morphologically this organism cannot be distinguished from various members of the hemophilus group

3 Culturally this organism resembles the Pasteurella group more closely than the hemophilus group but the fermentation reactions show essential differences

4 Pathogenicity of the organism described is much more marked for laboratory animals, including dogs, than organism of either the hemophilus or Pasteurella group compared with it, the most striking and constant lesion being a hemorrhagic interstitial pneumonia

5 Freshly isolated gram negative coccobacilli should be subjected to exhaustive cultural work and animal inoculation before classification is made

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HENRI FORD HOSPITAL

EMBRYONAL CARCINOMA OF THE TESTICLE*

By L. W. LARSON, M.D., BISMARCK, NORTH DAKOTA

A DESIRE to report six cases of embryonal carcinoma of the testicle and incidentally to review the voluminous literature on this subject has led to the preparation of this paper.

FREQUENCY

Testicular tumors are comparatively rare. Tanner¹ found that approximately 600 cases had been reported in the literature previous to 1922, that in 110,000 male admissions to the London Hospital, 65 had tumors of the testicle, that in 300,000 admissions, both male and female, to the Mayo Clinic, there were about 50 testicular tumors, and, that about one in 2000 male admissions (0.05 per cent) to a general hospital has this serious condition. Hinman² states that in 182,729 general male hospital admissions, there were 116 cases (0.063 per cent) of testicular tumor, and that there had been 649 cases reported in the literature. Morton³ found that 102 patients who had a tumor of the testicle had been operated upon up to the end of 1927, at the Mayo Clinic. The mortality statistics of the United States Bureau of Vital Statistics show that malignant tumors of the testicle constitute about 0.6 per cent of all malignant tumors in men.

HISTORY

A tumor of the testis was described in 1696 by St. Donat⁴ in which a rudimentary skull and the embryonic eyes of a parasitic fetus were found. Johnson,⁴ in 1856, was the first to ascribe a teridermal origin to certain tumors of the testicle. Langhans and Koehler,⁴ in 1887, with the aid of the microscope, ventured the opinion that most of the testicular tumors are teratomas.

ORIGIN

Ewing,⁵ in 1911, after having reviewed the literature thoroughly and having analyzed 19 cases, came to the conclusion that practically all testicular tumors are teratomatous in origin, that in those cases in which one type of tissue predominates, apparently at times to the exclusion of all other types of tissue, the true explanation lies in the assumption that the predominant tissue represents an overgrowth of that particular derivative, and the tumor, although teratomatous in origin, assumes the appearance of a unicellular type of tumor.

Chevassu,⁶ in a classic thesis, showed that 59 out of 120 cases of testicular tumor were of a solid medullary large celled type. He stressed the resemblance of these cells to the spermatocyte, and, assuming that they were

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derived from the cells of the seminal tubules, called the tumors "seminomes". The term "seminome" or "spermatocytoma" has since been used by Debernardi,⁷ Nicholson,⁸ Tanner,¹ Schultz and Eisendrath,⁹ Southam and Linnell¹⁰ in describing this particular type of tumor.

Ewing has remained steadfast in his belief that these tumors are teratomas mainly because he has often seen the characteristic appearing "seminome" cells in teratomas and in very early embryonal carcinoma, he has found minute traces of cartilage, endodermal alveoli and squamous epithelial cell groups. So, Ewing¹¹ is forced "to conclude that this common tumor of the testis is always a one sided development of a teratoma, and is not derived from the adult spermatoblasts." Hinman, et al. who have written voluminously on the subject and who favored the monodermal theory of origin, in a recent publication¹ report that they have found tissues of various types associated with typical seminomatous tissue and conclude that "the term 'seminome' or 'spermatocytoma' must therefore, be regarded as a misnomer, and the contention of Chiavassu is disproved in favor of Ewing's theory." In a Hunterian Lecture Cairns¹² substantiates the views of Ewing. Morris¹⁴ found typical "seminome" cells in a metastatic nodule in the lung from a case of teratoma of the testis and agrees with Ewing in that practically all tumors are essentially teratomas. The evidence therefore, seems fairly strong in favor of the theory that practically all testicular tumors arise from totipotent sex cells and are of teratomatous origin. The so called seminome is a modification of the carcinomatous degeneration found in most testicular tumors.

Recently, Stevens and Ewing¹⁵ have reported a case of adenocarcinoma of the testis, which they believe arose from the testicular tubules. They state that Gordon Bell had previously reported four such cases, in three of which Ewing concurs in the diagnosis. This type of tumor is characterized by an occurrence in a later decade of life and a slow growing affair with a relatively good prognosis. It would appear therefore, that there are two varieties of testicular carcinomas, the common type of embryonal carcinoma and the rare adenocarcinoma.

I believe that it is fairly well agreed (Ewing,⁴ Hinman¹²) that primary sarcoma of the testicle is comparatively rare. Dew¹⁶ believes that only about 2 per cent of testicular tumors can be regarded as primary sarcomas. Undoubtedly, a large proportion of the sarcomas reported in the literature is, in reality, carcinomas.

CLASSIFICATION

Granting, therefore, that all testicular tumors are teratomas, Ewing's¹⁷ classification seems to be quite acceptable especially to the clinician. He divides them into three main varieties:

- 1 Adult embryoma or teratoma
- 2 Embryoid, teratoid, or mixed tumors
- 3 Embryonal malignant tumors

The adult embryomas constitute a very small group of testicular tumors and are similar to the dermoids found in the ovary. The majority of them appear to be congenital, Cairns¹² finding an 84 per cent occurrence during the

METASTASES

All teratomas, whether mixed type or embryonal, are malignant and eventually metastasize. Extension is first along the spermatic lymphatics and veins. Secondary tumors in the lumbar nodes are common, Cairns¹³ reporting 29 out of 33 cases. An epigastric tumor involving the nodes of the celiac axis is often the first sign of metastasis. Metastases may extend up into the mediastinal and cervical nodes, where they may reach great size. Cases are on record in which the tumor spread continuously by means of the veins up to the heart. Embolic nodules in the lungs, liver, brain, kidneys, and stomach are more common as a result of venous passage. Invasion of the vertebrae with remittent paraplegia occasionally occurs. Likewise, involvements of one or both ureters followed by hydronephrosis and uremia have been reported.

DIAGNOSIS

It is not within the province of this paper to discuss the clinical diagnosis of testicular tumors. However, the diagnosis is primarily a matter of exclusion. In the differential diagnosis, inflammatory conditions, hydrocele, spermatocele, tuberculosis, gumma, and tumor must be considered.

PROGNOSIS

Malignant testicular tumors are recognized as among the most malignant neoplasms known. Chevassu⁶ reports 19 per cent living at the end of four years among 100 patients. Tanner¹ reports 465 followed cases. 377 or 81 per cent were dead from metastases, and 25 or 5.5 per cent were living and well four years or more after orchiectomy. He found that the mixed type of teratoma is more malignant (90 per cent) than the embryonal type (60 per cent).

The prognosis appears to be especially unfavorable in children. Kober (quoted by Kutzman²¹) found that 4 out of 10 patients died of metastases within one year of castration, one was living two months postoperative, and the other five had not been heard from. Steffen (quoted by Kutzman²¹) after reviewing 25 cases of his own and from the literature found that 13 patients were dead from recurrence or metastases within eleven months after operation, 7 were living, and of these 7, only 2 were well, and 7 were not heard from, therefore, of his followed cases, the mortality was 80 per cent.

TREATMENT

Several methods of treatment have been advocated. Simple orchiectomy will cure those cases in which metastasis has not occurred, but it is impossible to always detect the presence of early metastasis into the nodes usually involved. Since simple orchiectomy gives four-year cures in only 10 to 15 per cent of the cases, a more drastic treatment has been advocated by Hinman and others. In 1914, Hinman²⁴ reported a collection of 46 cases in which a radical operation had been performed. This procedure removes the lymph zones in the retroperitoneal and lumbar areas along the aorta and vena cava. The operative mortality was 11 per cent. Forty-six per cent were alive, 1 five years, 1 four years, 5 three years, 2 two years, and 11 one year or less following operation. There was a probable cure in at least 4 patients who

tubules, and Ewing maintaining that they are merely one sided developments of a preexisting teratoma. Whatever their point of origin, it is agreed that they constitute a distinct type of testicular tumor, both as to structure and malignancy.

ETIOLOGY OF TERATOMA TESTIS

Relation to Injury—In spite of the fact that most men have injured their testes at some time in their lives and relatively few develop neoplasms of these organs, a surprising number of patients presenting themselves with such tumors give a definite history of a previous injury. Cairns¹³ found 14 out of 79 patients (18 per cent), Tanner¹ found 22 in 100 (22 per cent), 3 of our 6 patients (50 per cent) gave a definite history of injury. Chevassu⁶ states that traumatism favors the growth of testicular tumors but believes that this theory has been overrated.

Relation to Position of the Testicle—The majority of writers agree that the undescended testicle is more prone to develop a teratoma than the normal organ. Arousseau² after summarizing 76 cases calculates that 10 per cent of the malignant tumors affect the abnormally placed testis and since only one out of a thousand testicles is found outside the scrotum the relative incidence of tumor in the undescended testicle is greater than in the normal organ. Lund³ says that malignant tumors of the normal testis are 103 times as frequent as in the testis in the abnormal position, but draws attention to the fact that only one in five hundred testes is abnormally placed. He quotes Bowring who states that malignancies of the undescended testicle constitute one third of testicular malignancies. Tanner¹ concludes that testes within the canal are more apt to become malignant. Cairns¹³ believes that the tendency of the undescended testicle to become malignant is overemphasized and quotes Howard, who after a wide experience has come to the same conclusion. Two of our 6 cases (33 per cent) were in undescended testes.

Age—Most testicular tumors occur during the ages of sexual activity. Tanner¹ found one under five years, none between six and seventeen years, 42 between eighteen and twenty nine years and 32 between twenty nine and thirty nine years. These tumors are comparatively rare in children. Only 5 of Chevassu's⁶ 61 cases of teratomas occurred in children under five years of age, and none of his 59 seminomas. Philipp collected 42 cases of testicular tumors in children from the literature up to 1908 (quoted by Kutzman²¹). Kelley (quoted by Kutzman²¹) states that a few dermoids have been reported in children. Our case of embryonal carcinoma in a child one year of age is probably the first to be reported. Kutzman and Gibson²² find only one case of seminoma in children reported in the literature, and that in a child seven years of age.

Testicle Involved—Cases reported in the literature show that the right and left testicles develop tumors with about equal frequency. Two of our 6 cases were in the right testicle and 4 in the left testicle.

Bilateral Occurrence—This is an extremely rare phenomenon, Chevassu⁶ finding only one in 128 patients.

METASTASES

All teratomas, whether mixed type or embryonal, are malignant and eventually metastasize. Extension is first along the spermatic lymphatics and veins. Secondary tumors in the lumbar nodes are common, Cairns¹³ reporting 29 out of 33 cases. An epigastric tumor involving the nodes of the celiac axis is often the first sign of metastasis. Metastases may extend up into the mediastinal and cervical nodes, where they may reach great size. Cases are on record in which the tumor spread continuously by means of the veins up to the heart. Embolic nodules in the lungs, liver, brain, kidneys, and stomach are more common as a result of venous passage. Invasion of the vertebrae with remittent paraplegia occasionally occurs. Likewise, involvements of one or both ureters followed by hydronephrosis and uremia have been reported.

DIAGNOSIS

It is not within the province of this paper to discuss the clinical diagnosis of testicular tumors. However, the diagnosis is primarily a matter of exclusion. In the differential diagnosis, inflammatory conditions, hydrocele, spermatocele, tuberculosis, gumma, and tumor must be considered.

PROGNOSIS

Malignant testicular tumors are recognized as among the most malignant neoplasms known. Chevassu⁶ reports 19 per cent living at the end of four years among 100 patients. Tanner¹ reports 465 followed cases. 377 or 81 per cent were dead from metastases, and 25 or 5.5 per cent were living and well four years or more after orchiectomy. He found that the mixed type of teratoma is more malignant (90 per cent) than the embryonal type (60 per cent).

The prognosis appears to be especially unfavorable in children. Kober (quoted by Kutzman²¹) found that 4 out of 10 patients died of metastases within one year of castration, one was living two months postoperative, and the other five had not been heard from. Steffen (quoted by Kutzman²¹) after reviewing 25 cases of his own and from the literature found that 13 patients were dead from recurrence or metastases within eleven months after operation, 7 were living, and of these 7, only 2 were well, and 7 were not heard from, therefore, of his followed cases, the mortality was 80 per cent.

TREATMENT

Several methods of treatment have been advocated. Simple orchiectomy will cure those cases in which metastasis has not occurred, but it is impossible to always detect the presence of early metastasis into the nodes usually involved. Since simple orchiectomy gives four-year cures in only 10 to 15 per cent of the cases, a more drastic treatment has been advocated by Hinman and others. In 1914, Hinman²⁴ reported a collection of 46 cases in which a radical operation had been performed. This procedure removes the lymph zones in the retroperitoneal and lumbar areas along the aorta and vena cava. The operative mortality was 11 per cent. Forty-six per cent were alive, 1 five years, 1 four years, 5 three years, 2 two years, and 11 one year or less following operation. There was a probable cure in at least 4 patients who

had invasion of the lumbar nodes at the time of operation. In a later publication Hinman⁵ reports a total of 79 cases in which a 30 per cent cure resulted from the radical procedure. Cairns¹³ states that out of 74 cases reported from the London Hospital, 55 of the patients had simple orcheectomy and 19 had the radical operation. There was no operative mortality. Recovery after orcheectomy was 33 per cent, and following the radical operation it was 31.2 per cent. This might be accounted for by the fact that those



Fig 1—Case 1 Gross specimen

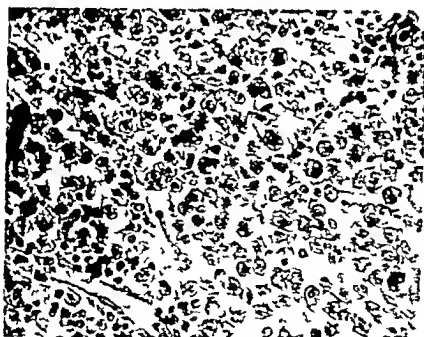


Fig 2—Case 1 Photomicrograph

patients in whom the radical operation was performed had mostly mixed tumors and therefore of greater malignancy than the single type cell tumors that comprised the majority of the patients given simple orcheectomy.

Coley²⁶ reports 78 cases of malignant tumors of the testicle treated with a combined vaccine of *Bacillus prodigiosus* and *Streptococcus erysipeloides*, in which 22 patients or 28 per cent survived more than two years.

Numerous writers recommend radium and x ray therapy, either alone or as an adjunct to surgery. Dean²⁷ reports 9 out of 13 patients with primary

operable tumors in which orchiectomy was not done, living from six months to five years one month after radium and deep x-ray therapy, 4 out of 16 patients with primary inoperable tumors with metastases living from six months to five years five months, all three patients with local recurrences after orchiectomy living from three years to ten years, 26 out of 81 patients (33 per cent) with inoperable recurrences and metastases following orchiectomy living from three months to nine years three months, 9 of whom lived



Fig 3—Case 2 Gross specimen

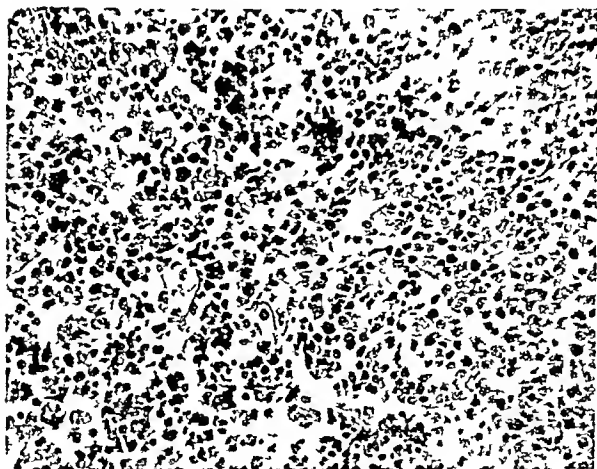


Fig 4—Case 2 Photomicrograph

over two years, and 8 out of 11 patients (72 per cent) who received prophylactic treatment after orchiectomy living from six months to nine years. Reports such as these tend to prove that radium and x-ray therapy have not only a palliative value, but probably vie with surgery in the average case. The embryonal types are more radiosensitive than the mixed teratomatous types.

Ewing, in a personal communication last year, doubted the value of the radical removal of regional lymph nodes and recommended the primary use

of x ray therapy, to be followed by orchectomy and then more x ray therapy Higgins²⁸ advocates the same procedure

Our procedure has been the use of x ray therapy following orchectomy, which I believe is also followed at the Mayo Clinic

CASE REPORTS

CASE 1—Henry W, aged thirty eight, German farmer married Came to our Clinic May 20, 1921, because of an undescended left testicle and a tumor in the left inguinal region Past history was negative Present illness began eight weeks ago when tumor mass in left

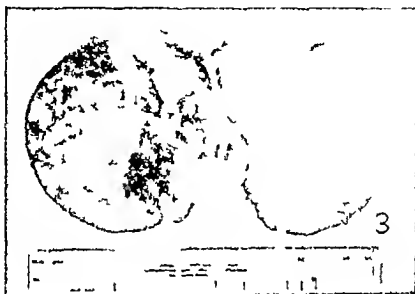


Fig 5—Case 3 Gross specimen.

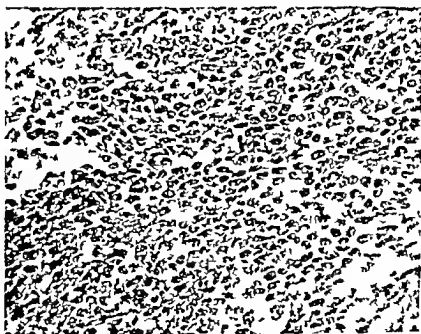


Fig 6—Case 3 Photomicrograph

inguinal region began to appear The tumor had grown progressively and was so painful that he could not work

Physical examination revealed an undescended left testicle and a large tumor in the left inguinal region The general physical examination was negative No evidence of metastasis could be demonstrated Orchectomy was done May 28 1921

The specimen consisted of a well encapsulated soft tumor which on cut section, presented a pale pink solid surface The epididymis was not involved Paraffin sections revealed an embryonal carcinoma in a lymphoid stroma No other tissue elements could be recognized

He was given light x-ray therapy to four areas in the left inguinal region on June 30, 1921. A letter from the patient stated that he was well on May 28, 1929, eight years after operation.

CASE 2—James R, aged thirty nine, Russian, farmer, married. Came to our Clinic March 4, 1925, complaining of a swollen left testicle. Past history was negative. Present illness began ten months ago when he was kicked by a horse. The left testicle was injured and began to swell very soon after the accident. There was pain in the testicle for some time after the injury, but lately the pain has subsided. The testicle has grown progressively larger since the injury. No other complaints.

Physical examination revealed a robust well appearing male. Except for an advanced

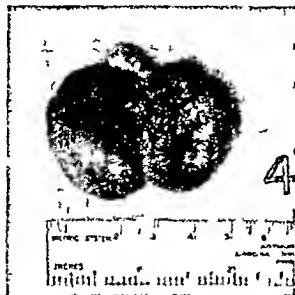


Fig 7—Case 4 Gross specimen

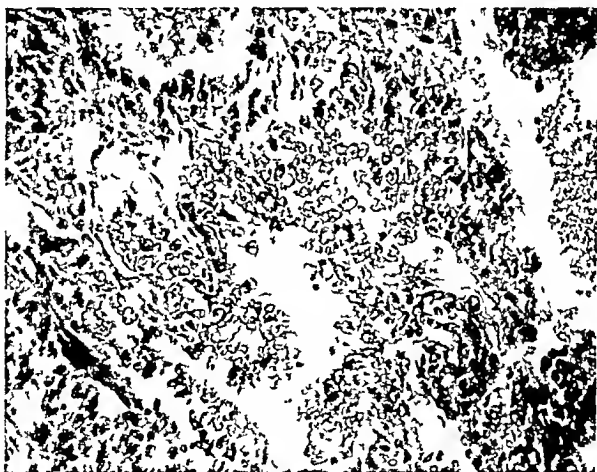


Fig 8—Case 4 Photomicrograph

pyorrhea and several infected teeth, the general physical examination was negative. The left testicle was enlarged to about twice the normal size, and was firm and smooth. Orchiectomy was performed March 5, 1925.

The specimen consisted of a large well encapsulated tumor measuring 5 by 6 by 8 cm. The testicular tissue could not be recognized grossly. The epididymis appeared uninvolved. Cut section presented a spongy, lobulated, yellowish white surface of similar character throughout.

Paraffin sections revealed the typical embryonal type of carcinoma in a lymphoid stroma. No other tissues could be recognized.

The patient was given deep roentgen therapy to the testis, groin and mediastinum, beginning March 9, 1925, and seven times thereafter at irregular intervals until May 27, 1926.

From January until June 1926 he complained of much abdominal and left kidney pain. He died September 3, 1926 eighteen months after operation.

CASE 3—Joe C. L. aged thirty single, laborer. Came to our Clinic March 13 1926 complaining of backache and a rupture. Past history was negative except that he had always had a left sided rupture. No history of injury. The present illness began three weeks ago with backache and pain in the left side. There was no fever nor chills. Since the pain began the rupture had been 'out' and did not go back again.

Examination showed negative findings except for a questionable left inguinal hernia and a left hydrocele.



Fig 9—Case 5 Gross specimen

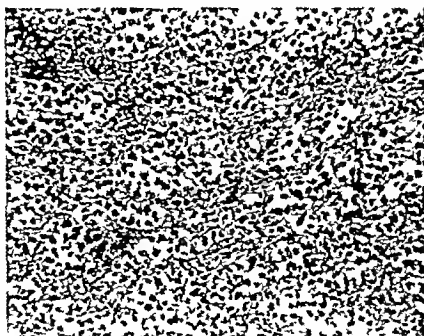


Fig 10—Case 5 Photomicrograph

Operation was performed March 16 1926 and about an ounce of straw colored fluid was found in the left tunica. The testicle was enlarged to the size of an orange. Orchiectomy was done and a left inguinal hernia repaired.

The specimen consisted of an egg shaped tumor measuring 6 by 6 by 9 cm. All testicular tissue appeared to be destroyed. The tumor was well encapsulated and on cut section presented a lobulated grey surface with small areas of hemorrhage and degeneration. The epididymis was not involved.

Paraffin section revealed an embryonal carcinoma in a lymphoid stroma. No other tissues could be recognized.

He was given deep roentgen therapy, anterior and posterior, to the lower abdomen and left groin, on March 25, 1925. He left the hospital April 4, 1925, and returned April 27th complaining of pain in the left renal area. At this time he was given deep x ray therapy to the lower abdomen and also to the left renal area. The patient left for his home in Illinois in May, 1925. He entered a hospital there, but was not given x ray treatment. A letter from his physician stated that he died November 1, 1925, six and one half months after operation.

CASE 4—Arnold W, aged one year, was brought to us June 4, 1926 because of a progressive increase in the size of the left testicle since birth. History negative. Normal birth. Weight 9 pounds at birth. Breast fed. Had done well.

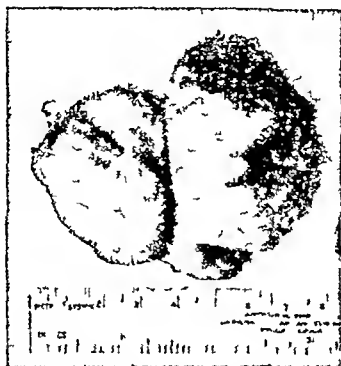


Fig 11—Case 6 Gross specimen

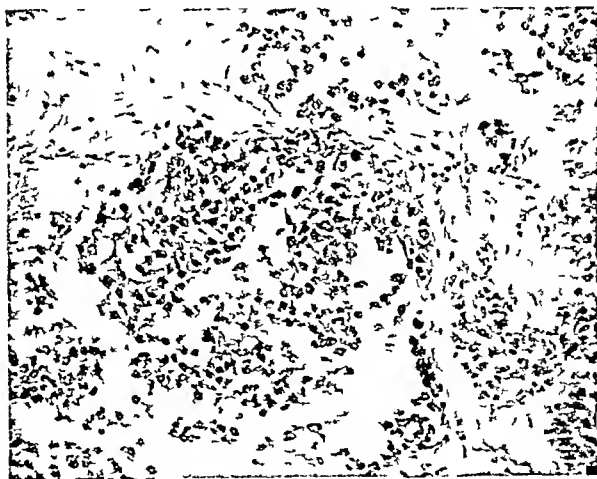


Fig 12—Case 6 Photomicrograph

Examination revealed signs of rickets. A hard tense tumor about the size of a small hen's egg was present in the left side of the scrotum. It did not transilluminate light. No areas of softening or fluctuation could be detected.

Orchectomy was done June 5, 1926.

The specimen measured $2\frac{1}{2}$ by 3 by 4 cm. The entire testicular substance was replaced by a solid pearl colored tumor which was well encapsulated. There were no areas of softening or necrosis. One small area appeared to be hemorrhagic. The epididymis was not involved.

Paraffin sections revealed cords and nests of embryonal carcinomatous tissue in a lymphoid stroma. No other tissue elements could be recognized.

No x ray or radium treatment was given. A letter from the father stated that the child was living and well June 5, 1929 three years after operation.

CASE 5—Richard P. aged thirty, American, miner married. Consulted our Clinic on January 14, 1929 complaining of a lump in the right groin. First noticed a painless lump about the size of an almond in the right groin in 1917. A year ago the lump began to increase in size. Three months ago he slipped and strained the right limb and from then on the lump slowly increased in size. Last week following a strain while lifting the lump increased in size to that of two large thumbs. Had never had pain in the lump until the past week and since then it has pained him only on exertion. The past history was essentially negative.

Physical examination was negative except for a freely movable painless mass the size of a small potato in the right groin. The right testicle was not in the scrotum. The left testicle was normal.

Operation was performed January 15, 1929. A tumor was found in the right inguinal region lying just below the external inguinal ring. The mass removed by the multitherm electrocautery knife and the hernia repaired.

The specimen measured $2\frac{1}{4}$ by 5 by $3\frac{1}{2}$ cm. The enlargement was symmetrical, the surface was smooth, and the epididymis was normal. The capsule was smooth and intact. The corpus was firm and somewhat nodular and on cut section presented a lobulated moist greyish white surface on which numerous fine connective tissues could be seen. There was no gross evidence of degeneration nor could any normal testicular tissue be recognized.

Paraffin sections showed a typical embryonal carcinoma in a lymphoid stroma. No other tissue elements could be recognized.

He was given a course of x ray therapy on January 24, 1929. He was living and well on June 15, 1929, six months after operation.

CASE 6—Godolph R., aged twenty four, German single farmer. Consulted our Clinic on March 16, 1929 complaining of an enlargement of the right testicle. He first noticed the enlargement seven months ago. Has no pain in the testicle except on manipulation. Has doubled in size during the past six weeks. No other complaints. Past history revealed lung trouble for a year nine years ago. Had a cough hemoptysis etc. but did not consult a physician so he does not know whether he had tuberculosis or pneumonia. Has also had much trouble with pes planus.

Physical examination showed a well developed and well nourished young male. There was a submaxillary and cervical adenopathy present, a compensated mitral insufficiency, intact inguinal rings, pes planus, and a firm smooth enlargement of the right testicle. The lungs were negative.

Orchectomy was performed March 18, 1929.

The specimen measured $3\frac{1}{2}$ by 4 by 7 cm. It was very firm and its capsule was smooth and vascular. The epididymis was smooth and normal, grossly. Cut section through the corpus showed almost the total testicular substance with the exception of a small area in the superior portion at the periphery, replaced by a greyish white diffuse tumor which had a moist surface.

Paraffin sections showed an embryonal carcinoma with numerous strands of fine connective tissue in which a few lymphocytes could be found.

A series of x ray treatments were given from March 27 to 29, 1929 and a second series on April 30, 1929. He was living and well on June 18, 1929 three months after operation.

SUMMARY OF CASES REPORTED

An analysis of our cases reveals some interesting observations (see Figs 13 and 14). The ages of the patients ranged from one year to thirty nine years, four occurring during the third decade and one during the second decade and one in infancy. Three (50 per cent) patients gave a definite history of a previous injury to the organ. The length of time that the patients

had been conscious of an abnormality varied from three weeks to ten or twelve months. Two of the patients complained of constant pain in the testicle, two experienced pain only when the organ was handled or in the least way traumatized, one had had pain but was free from pain when he consulted us, and the infant had never complained of pain. Four occurred in the left testicle and two in the right testicle. In three cases (50 per cent) a hydrocele complicated the tumor. Two cases (33 per cent) were in undescended testes and a like number were associated with an inguinal hernia, in one case (Case 5) both the abnormal position of the testicle and an accompanying hernia being present.

CASE	AGE	DURATION	PAIN	TRAUMA	TESTICLE INVOLVED
1	38	8 wk	+	-	L
2	39	10 mo	Pain after trauma—subsidised	Kicked by horse 10 mo ago	L
3	30	3 wk	+	-	L
4	1	10 12 mo	-	-	L
5	30	Lump groin 12 yr Increased size 3 mo ago	On exertion only	In childhood, also 3 mo ago	R
6	24	7 mo	Only on manipulation	While riding horse	R

Fig 13—Analysis of case reports

CASE	HERNIA	TESTICLE UNDESCENDED	WITH HYDROCELE	X RAY THERAPY	RESULT
1	-	+	+	+	Living 8 yr
2	-	-	+	+++	Died 18 mo
3	+	-	+	Large doses	Died 6½ mo
4	-	-	-	+	Living 3 yr
5	+	+	-	+	Living 5 mo
6	-	-	-	+	Living 3 mo

Fig 14—Analysis of case reports

The mortality to date has been 33 per cent, one patient living six and one-half months and one patient eighteen months after orchiectomy. In both of the fatal cases, the patients obtained intensive x-ray therapy. The patient that has lived the longest (eight years) received only two sittings of small doses of roentgen therapy, while the infant, who has lived three years, received no x-ray treatment. Cases 5 and 6 have received massive doses of x-ray therapy, but they are both such recent cases that conclusions could not be fairly drawn from them. Thus, a measure of the efficacy of x-ray treatment from a study of our cases cannot be determined. The only logical conclusion must be that in the fatal cases, metastases had taken place by the time operation was performed, while in the apparently cured cases (Cases 1 and 4) the neoplasm was limited to the testicle.

COMMENT

Because of the nature of the organ from which tumors of the testicle arise, it seems reasonable to suppose that most of them would have a complex structure. The evidence set forth by Ewing, Hinman, and others seems

conclusive that they are all essentially teratomatous. We have been unable to find any other derivatives than the embryonal type of carcinomatous cells in our primary tumors. However, we have not had the opportunity of making postmortem examinations of any of our cases and thereby determine the type of tissue found in all the metastatic nodules. The important consideration regardless of varying opinions as to tissue derivatives, classifications, nomenclature, etc., is that the type of tumor herein reported constitutes a distinct clinical entity with a greater radiosensitivity and therefore a better prognosis than the tumor that is obviously teratoid in structure.

The question of the etiology of tumors is always an interesting one. All that we have been able to suggest with regard to testicular neoplasms are contributing factors only. Just what influence trauma, position of the testicle, previous infections, etc., have on the subsequent development of a neoplasm it is most difficult to evaluate from the data at hand. Certainly age must be an important factor since most of the testicular tumors develop between the ages of eighteen and forty years. This is the period of greatest sexual activity in the average male and it seems reasonable to suppose that this factor is a very important predisposing one. And yet, the occurrence of a tumor in a child one year of age, such as the one herein reported, which is similar to the type of tumors occurring in mature men, would appear to entirely overthrow the theory that sexual activity is the most important contributing factor. Not until the causative agent or agents are discovered will we be able to properly evaluate the meager information we have as to etiology.

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DISCUSSION

Dr Charles R. Drake—Tissue pathology is of special interest to me, and this particular type of pathology has been more or less confusing. I feel that we must be careful and not be too dogmatic in our terminology, so that the more I have studied these types of tumors the more I have been inclined to call them "malignant tumors of the testicle" rather than to classify them in any particular way. Wherever there are epithelial cells, there also you can have carcinoma no matter where it is. As to the two groups of pictures of Dr. Larson's, they were individually more or less uniform in character. One group showed the variation in type of growth and emphasized the fact of not being too dogmatic about terminology. Epithelial cells may change from the normal, and when the epithelial cells become malignant, they do not continue in their normal type of growth. Not only do they change in the methods of growth but also in morphology and therefore can take on multiple types of shape and character as shown by these slides. A year or two ago I saw a tumor of the testicle about 5 cm. in diameter which was typically adenocarcinoma. These are somewhat rare. There was no suspicion of any other type of growth in it. This was confirmed by other clinics. All these tumors are malignant or nearly so. I cannot help but feel like emphasizing the necessity in not being too dogmatic in our classification of these tumors and that a carcinoma may originate in any part of the body, at any time, from any source, wherever there is epithelial structure.

Dr Ezra E. Bohn—I have personally seen quite a number of Dr. Hinman's slides and those of Dr. Rusk. I think that I probably have seen two or three tumors which were cut up to small pieces and practically entirely blocked and nothing could be found but an adenocarcinomatous arrangement. It is interesting to note that the author found a large number of rest cells embryonic in type. It might be that these are responsible for some of the tumors.

Dr Roy W. Hammacl—There is just one point which I wish to bring up, that is the question of the relation of trauma to etiology as it comes up under the Workmen's Compensation Act. Of course, the whole subject of the relation of trauma to tumors comes up there also. Everyone can recall an injury at some time or other to the testicle, and the workmen who develop malignant tumors are no exception. When these cases come before the Industrial Accident Commissions they must attempt to settle the question. Now, it seems to me that the literature is indefinite on the subject as I suppose it must be. I have just reviewed fourteen or fifteen papers published in the last few years, and I think there were only four in which this question was discussed. All of these rather tended to deny the idea that trauma is a factor of importance. When these cases do come before the Industrial Accident Commissions, the pathologist is usually called upon to express an opinion either for the insurance company in defense, or else by the beneficiary. What is he going to say?

Dr Leonard W. Larson—I think that Dr. Drake's remarks about being conservative in the classification of testicular tumors is well taken. I do not wish to give the impression that this particular type of testicular tumor is the only type found. We have had five cases since I came to Bismarek, and I find record of one before that time, making a total of six. Thus, of course, is merely a coincidence, and it shows that reporting a small series of cases does not mean so much. However, it is interesting to note that we have seen this type of tumor in six instances during a five-year period. If you will investigate the article written by Hinman recently you will find a classification of testicular tumors that covers about four pages. I think Ewing's attempt to put them into a few simple types is a very good one.

I was much interested to hear Dr. Bohn's statement that he had seen a pure adenocarcinoma. I am sure Dr. Ewing would be much interested in seeing Dr. Bohn's case because when I talked to him over a year ago he was very enthusiastic because he had found such a case. He has reported that case within the last month or so.

The relation of trauma to the etiology of tumors is a very important one. I do not know what the medical profession is going to do about it. We see a great number of cases in which this question comes up because the Workmen's Compensation Bureau is located in our city. Recently, an elevator operator, who had fallen and injured his chest came in with

an abscess of the chest which had developed at the point of injury some time after the accident. On examination we found that he had an old tuberculosis in both lungs and that the abscess was a tuberculous abscess. Of course he and his friends are going to prove to the Workmen's Compensation Bureau that the injury caused the abscess. There should be some way of getting together on these things.

Dr O A Brinc—There is no use saying this matter has all been cleared up. It is still somewhat confusing and yet I think that the whole thing has been to quite a large extent clarified and I think that the idea that we are dealing primarily with mixed tumors is becoming gradually accepted. The most difficult thing is to hit upon a real good name to call them. Probably one is as good as the other. Teratoma is all right except that they do not resemble the average teratoma. It certainly is true that the epithelial tissue in these neoplasms is the important thing. The other tissue does not really enter in very much. The greatest plea that can be made is to simplify the present classification and instead of the large number of terms now being used to describe these tumors perhaps one name can be used for all of them.

OXALIC ACID AS A REAGENT FOR ISOLATING TUBERCLE BACILLI AND A STUDY OF THE GROWTH OF ACID-FAST NONPATHOGENS ON DIFFERENT MEDIUMS WITH THEIR REACTION TO CHEMICAL REAGENTS*

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I N DETERMINING the value and limitations of a method for isolating tubercle bacilli from contaminated tuberculous materials for diagnostic purposes, consideration must be given to many factors which may contribute to the simplicity and accuracy of the procedure for practical purposes. Earlier studies by us,¹ reported before the American Society of Clinical Pathologists as part of the outline of the Research Committee, have established that an appropriate culture method could be used advantageously in place of guinea pig inoculation for the diagnosis of tuberculosis when the bacilli were not found to be present in the ordinary stained smear examined microscopically.² The method essentially consists in destroying the contaminating microorganisms in the tuberculous material by the addition of an equal volume of 6 per cent sulphuric acid and after thorough mixing, incubating for thirty minutes, diluting the acid with sterile saline and planting the sediment on a 6 per cent glycerol water crystal violet potato cylinder medium.³ This medium was found in comparative quantitative tests to be the best nutrient medium, of all those tested, for the purpose of supporting the growth of tubercle bacilli when present in only small numbers in the tuberculous materials, as well as being best suited, due to the crystal violet present, to suppress the growth of such rapidly growing microorganisms as may have survived the sulphuric acid treatment.

There are certain undesirable features to the use of sulphuric acid, such as its marked avidity for water which makes likely an alteration in concentration after long standing, especially in strong stock solution. Its corrosive action on the hands and clothing is also a disadvantage to its use. In order, if possible, to find a reagent not possessing these undesirable features, a survey of other acids was made with the result that oxalic acid, obtainable in pure crystalline form and, therefore, of definite chemical composition, proved encouraging in that it gave promising results in preliminary tests which indicated a higher yield of cultures as well as a lower percentage of contamination.

I A COMPARISON OF OXALIC ACID AND SULPHURIC ACID AS A REAGENT FOR DESTROYING CONTAMINATORS

In an effort, therefore, to improve and simplify the culture method for tubercle bacilli as far as possible the following experiments were performed in elaboration of earlier encouraging preliminary tests. For the purpose of

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From the Research Department National Jewish Hospital at Denver Colorado

comparing the efficiency of oxalic acid as compared to sulphuric acid sixteen positive tuberculous sputums were obtained and after thorough mixing were divided into one cubic centimeter portions, placed in 15 c.c. graduated centrifuge tubes each specimen treated with an equal volume of different concentrations of oxalic acid from saturated to 2 per cent solution and for the purpose of control a similar one cubic centimeter specimen was treated in the usual prescribed manner with an equal volume of 6 per cent sulphuric acid. After incubating the well mixed acid treated specimens for thirty minutes at 37° C, with occasional shaking during this time each was diluted by the addition of 10 c.c. of sterile 0.9 per cent saline solution and after centrifugation the sediment was planted on tubes of crystal violet potato cylinder medium and read after regular intervals of incubation at 37° C. The findings resulting from this comparative study are recorded in Table I.

TABLE I

A COMPARISON OF OXALIC ACID AND SULPHURIC ACID AS REAGENT IN THE TREATMENT OF POSITIVE TUBERCULOUS SPUTUMS FOR ISOLATING TUBERCLE BACILLI

REAGENT USED	CULTURE RESULTS IN PER CENT	
	POSITIVE TUBERCLE BACILLUS ISOLATIONS†	CONTAMINATIONS
6 per cent Sulphuric Acid	78	28
Saturated Oxalic Acid (about 9.5 per cent)‡	86	15
7 per cent Oxalic Acid	85	14
5 per cent Oxalic Acid	88	15
2 per cent Oxalic Acid	66	45

The percentage results were calculated from the number of culture tubes presenting positive cultures of tubercle bacilli or contaminations as compared to the total number of tubes used in each test. There were five tubes planted from each sputum in each case making a total of eighty tubes for each reagent.

†On the basis of sputums alone the results were 100 per cent in that every sputum gave at least one positive culture for tubercle bacilli even including those treated with the 2 per cent oxalic acid reagent.

‡International Critical Tables published for the National Research Council by McGraw Hill Book Company 4 250 1928 gives 9.52% oxalic acid ($C_2H_2O_4 \cdot 2H_2O$) soluble at 20° C.

It is to be noted from an examination of the results recorded in Table I that the findings with saturated or 7 or 5 per cent oxalic acid were almost identical so far as percentage isolations of pure cultures and percentage contaminations were concerned while 2 per cent oxalic acid proved to be decidedly less serviceable both in destroying contaminants as well as providing pure cultures of tubercle bacilli. In concentrations of 5 per cent to a saturated solution oxalic acid also proved to be more satisfactory than 6 per cent sulphuric acid, in that a definitely lower percentage contaminations resulted from its use as well as a greater percentage of positive cultures of tubercle bacilli being yielded.

The foregoing results with positive sputums suggested a greater germicidal action of the oxalic acid in 5 per cent to saturated solution (equal volume being added) toward the contaminants as well as a reduced toxicity toward the tubercle bacilli as compared with an equal volume of 6 per cent sulphuric acid in sputums. It therefore appeared advisable to determine further whether any difference existed in control experiments with seedings with pure suspensions of tubercle bacilli in tuberculostatic tests. For this

purpose tubercle bacilli were planted in graded amounts upon tubes of crystal violet potato medium to which had been added one cubic centimeter of 6 per cent glycerol water containing varying amounts of oxalic acid from 0.0001 per cent to 5 per cent. The seedings with finely divided suspensions of virulent human tubercle bacilli ("Gluckson") ranged from 0.000,001 to 10 mg. The growth on this medium at 37° C using glycerol water crystal violet potato cylinder medium as control was read at regular intervals and recorded. The results after eight weeks incubation are recorded in Table II.

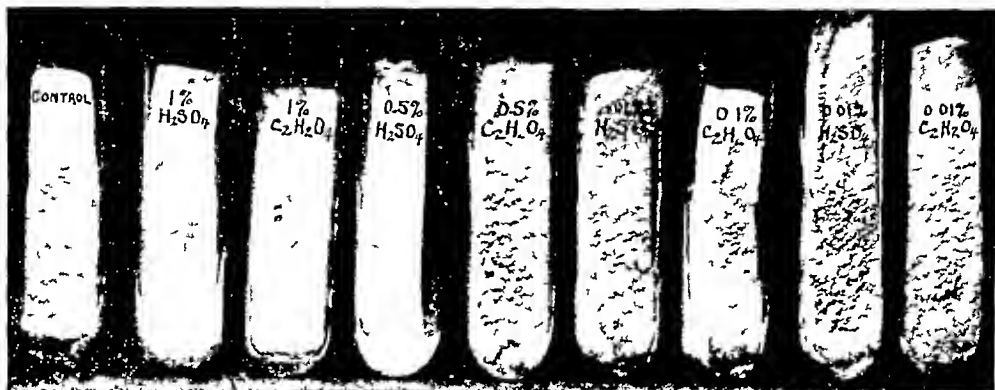


Fig. 1—Tuberculostatic action of oxalic acid ($\text{C}_2\text{H}_2\text{O}_4$) in glycerol water crystal violet potato cylinder medium compared with that of sulphuric acid (H_2SO_4). The medium was planted with a suspension of virulent human tubercle bacilli (Gluckson) containing one milligram per cubic centimeter and incubated for three weeks at 37° C. The concentration of oxalic or sulphuric acid in the glycerol water added to the crystal violet potato cylinder is designated for each tube in the photograph. Note the decided inhibition of growth with the 0.5 per cent sulphuric acid which was hardly appreciable with the 0.5 per cent oxalic acid at this time.

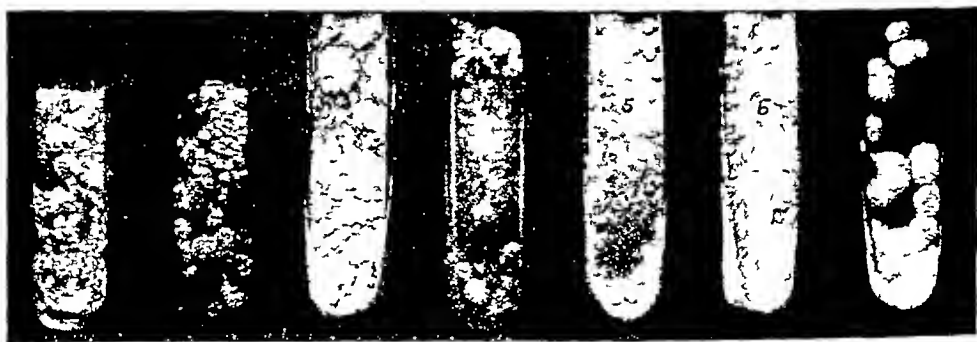


Fig. 2—Growth of smegma bacilli on different mediums planted with a suspension containing 0.0001 mg per c.c. and incubated fourteen days at 37° C. Note growth on all the mediums slightly less so on Long's nonprotein agar medium. From left to right the tubes of medium are: (1) glycerol water potato cylinder medium, (2) glycerol water crystal violet potato cylinder medium, (3) medium P (25 per cent ground potato, 2½ per cent glycerol and 1½ per cent agar), (4) Long's nonprotein medium, (5) Petroff's gentian violet egg medium, (6) Dorset's egg medium and (7) 5 per cent glycerol broth agar.

It is clearly evident from the findings recorded in Table II that oxalic acid in 0.1 per cent concentration in the glycerol water exerts no tuberculostatic action, while concentrations of 1 per cent or more exercise a definite retarding influence upon the growth of the tubercle bacilli on the crystal violet potato medium. Now if we apply this information to determine whether or not the oxalic acid used to destroy the undesirable contaminants in tuber

enous materials exerts a detrimental influence upon the bacilli, it is to be noted that the 5 per cent concentration is reduced to 25 per cent when added to an equal volume of the specimen being tested, and on dilution with 10 c c of 0.9 per cent sodium chloride solution, the concentration of oxalic acid becomes about 0.5 per cent which suffers a reduction to less than 0.1 per cent in being added as a few drops of residue planted on the glycerol water crystal violet potato cylinder medium containing, besides the potato, 15 c c of 6 per cent glycerol water. The fact that the oxalic acid is innocuous to the growth of the tubercle bacilli when used in concentrations even to an equal volume of saturated solution is also borne out by the tests with sputums recorded in Table I.

TABLE II

TUBERCULOSTATIC ACTION OF OXALIC ACID ON VIRULENT HUMAN TUBERCLE BACILLI GROWN ON GLYCEROL CRYSTAL VIOLET POTATO CYLINDER MEDIUMS AFTER EIGHT WEEKS AT 37° C

AMOUNT OF OXALIC ACID IN 1 C C 6 PER CENT GLYCEROL ADDED TO CRYSTAL VIOLET POTATO CYLINDER MEDIUM	AMOUNT OF SUSPENSION IN NO. OF TUBERCLE BACILLI PER C C USED FOR SEEDING CULTURE TUBES		
	10	0.0001	0.000001
Control Glycerol Crystal Violet Potato Cylinder Medium	4*	2	1
5 per cent Oxalic Acid	0	0	0
1 per cent Oxalic Acid	2	1	0
0.1 per cent Oxalic Acid	4	2	1
0.001 per cent Oxalic Acid	4	2	1
0.0001 per cent Oxalic Acid	4	2	1

The amount of growth obtained on the medium is arbitrarily graded from 0 = no appreciable macroscopic growth visible to 4 = a heavy and profuse growth over the entire medium.

In order to carry the comparison of tuberculostatic action of the oxalic acid and the sulphuric acid out more exactly another experiment was performed in which these acids in graded amounts were added to the 6 per cent glycerol water which was then used in the previously prescribed amounts (1 to 1½ c c) incorporated with the crystal violet potato cylinder in a 6 by ¾ inch bacteriologic culture tube. These culture tubes were then planted with graded amounts of suspensions of virulent human tubercle bacilli (Gluckson) incubated at 37° C and read at weekly intervals. As control the 6 per cent glycerol water crystal violet potato cylinder medium was used. The results of this study are recorded in Table II A giving the readings after eight weeks' incubation.

The findings recorded in Table II A indicate that the sulphuric acid in like concentrations in the glycerol water added to the crystal violet potato cylinder mediums has a greater tuberculostatic action than the oxalic acid which is distinctly noticeable in the concentrations of 0.5 per cent and greater. Oxalic acid exerted no effect in 0.5 per cent concentration and both acids were without appreciable effect upon the growth of the virulent human tubercle bacilli in the concentration of 0.1 per cent or less.

Experiments are now in progress comparing oxalic and sulphuric acids for isolating tubercle bacilli from urine and animal tissues.

In tuberculostatic tests with virulent human tubercle bacilli as test organism and glycerol water crystal violet potato cylinder medium as nutrient, sodium oxalate proved far less toxic than oxalic and sulphuric acid in that even a one per cent concentration of this salt in the 6 per cent glycerol water exerted no retarding influence upon the growth of the bacilli while a 5 per cent concentration exerted only a slight retarding effect. However, sodium oxalate incorporated in 5 per cent glycerol broth again was decidedly bacteriostatic in concentrations over one per cent but exerted no effect in 0.1 per cent concentration.

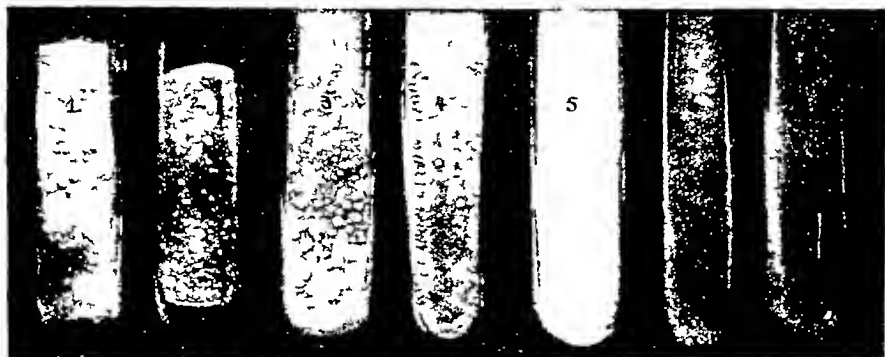


Fig 3—Growth of virulent avian tubercle bacilli on different mediums planted with a suspension containing 0.000.01 mg per c.c. and incubated five weeks at 37° C. Note growth on all the potato mediums and on Petroff's medium, doubtful growth on Dorset's medium and no growth on Long's medium or glycerol agar. From left to right the tubes of medium are (1) glycerol water potato cylinder medium (2) glycerol water crystal violet potato cylinder medium (3) medium P (25 per cent ground potato 2½ per cent glycerol 1½ per cent agar) (4) Petroff's gentian violet egg medium (5) Dorset's egg medium (6) Long's nonprotein agar medium and (7) 5 per cent glycerol broth agar.



Fig 4—Growth of acid-fast glass bacilli on different mediums planted with a suspension containing 0.000.001 mg per c.c. and incubated eight weeks at 37° C. Note growth on all the potato and egg mediums but none on the glycerol agar or Long's medium at this time. From left to right the tubes of medium are (1) glycerol water potato cylinder medium (2) glycerol water crystal violet potato cylinder medium (3) medium P (25 per cent ground potato 2½ per cent glycerol 1½ per cent agar) (4) Petroff's gentian violet egg medium (5) Dorset's egg medium (6) Long's nonprotein medium and (7) 5 per cent glycerol broth agar.

In view of the fact that in earlier studies with virulent human tubercle bacilli the effect of acetic acid as a reagent for destroying undesirable contaminants had not been recorded and considering the interest at this time of the effect of this acid upon human tubercle bacilli as compared to the effect

on other nonpathogenic and pathogenic acid fast bacilli the following experiment is now recorded. According to the usual procedure used by us for testing the effects of various concentrations of a reagent upon both the undesirable contaminants and the tubercle bacilli in a pathologic specimen eight positive sputums were appropriately prepared and treated with an equal volume of varying concentrations of pure acetic acid from 2 to 20 per cent (by weight), and as control like specimens were treated with an equal volume of 6 per cent

TABLE II A

TUBERCULOSTATIC ACTION OF OXALIC ACID IN GLYCEROL WATER CRYSTAL VIOLET POTATO MEDIUM COMPARED WITH THAT OF SULPHURIC ACID AFTER EIGHT WEEKS INCUBATION AT 37° C

PER CENT OXALIC OR SULPHURIC ACID IN 6 PER CENT GLYCEROL WATER ADDED TO MEDIUM	AMOUNT OF VIRULENT HUMAN TUBERCLE BACILLI (OLUCKSON) IN MG PER CC IN SUSPENSION USED FOR SEEDING CULTURE TUBES		
	10	0.0001	0.000001
Control glycerol water crystal violet potato cylinder medium	4*	2	1
30 per cent Oxalic Acid	0	0	0
30 per cent Sulphuric Acid	0	0	0
10 per cent Oxalic Acid	2	1	0
10 per cent Sulphuric Acid	1	0	0
0.5 per cent Oxalic Acid	4	2	1
0.5 per cent Sulphuric Acid	3	1	0
0.1 per cent Oxalic Acid	4	2	1
0.1 per cent Sulphuric Acid	4	2	1

*The amount of growth obtained on the medium is graded from 0 = no appreciable macroscopic growth visible to 4 = a heavy profuse growth. On concentration less than 0.1 per cent of added oxalic or sulphuric acid there occurred no retardation of growth.

TABLE II B

THE EFFECT OF ACETIC ACID UPON THE MICROORGANISMS IN TUBERCULOUS SPUTUMS*

REAGENT USED FOR THE PRELIMINARY TREATMENT OF SPUTUMS BEFORE PLANTING ON GLYCEROL WATER CRYSTAL VIOLET POTATO CYLINDER MEDIUM	PURE CULTURES OF TUBERCLE BACILLI		UNDESIRABLE GROWTHS	
	NUMBER OF TUBES POSITIVE	PER CENT OF TOTAL TUBES POSITIVE†	NUMBER OF TUBES CONTAMINATED	PER CENT OF TUBES CONTAMINATED
Control 6 per cent Sulphuric Acid	30	75	10	25
20 per cent Acetic Acid	0	0	0	0
10 per cent Acetic Acid	3	8	5	12
7 per cent Acetic Acid	3	8	3	8
5 per cent Acetic Acid	2	5	7	18
2 per cent Acetic Acid	1	2	11	28

Eight tuberculous sputums positive by microscopic examination were used in this study.

†A total of forty tubes of glycerol water crystal violet potato cylinder medium were used for the tests for each reagent and the percentages are figured on this basis.

sulphuric acid and incubated (thirty minutes at 37° C) diluted (with 0.9 per cent saline solution) the centrifugated residues planted on five tubes of glycerol water crystal violet potato cylinder medium for each dilution of acid used and for each specimen of sputum being tested. The results of these tests are briefly recorded in Table II B.

It is evident from the results of the study with acetic acid recorded in Table II B that acetic acid even in concentrations as low as 2 per cent added in equal volume to tuberculous sputums and incubated for thirty minutes has a deleterious effect upon the human tubercle bacilli present in the sputums.

II THE GROWTH OF ACID-FAST NONPATHOGENS ON VARIOUS NUTRIENT MEDIUMS AND THEIR REACTIONS TO VARIOUS CHEMICAL REAGENTS

Within the past few years Sewall and De Savitsch⁴ reported on an interesting phenomenon of antagonism occurring between smegma bacilli and tubercle bacilli, and Dr Sewall suggested the desirability of learning more about the reactions of the nonpathogenic acid-fast bacilli, and particularly the smegma bacillus, to various chemical reagents and especially so since the usual



Fig 5—Growth of acid-fast butter bacilli on different mediums planted with a suspension containing 0 000 001 mg per cc and incubated four weeks at 37° C. Note growth on all the potato and egg mediums at this time and its absence on glycerol agar and Long's medium. From left to right the tubes of medium are (1) Long's nonprotein agar medium (2) glycerol water potato cylinder medium (3) glycerol water crystal violet potato cylinder medium (4) medium P (25 per cent ground potato 2½ per cent glycerol 1½ per cent agar) (5) Petroff's gentian violet egg medium (6) Dorset's egg medium and (7) 5 per cent glycerol broth agar

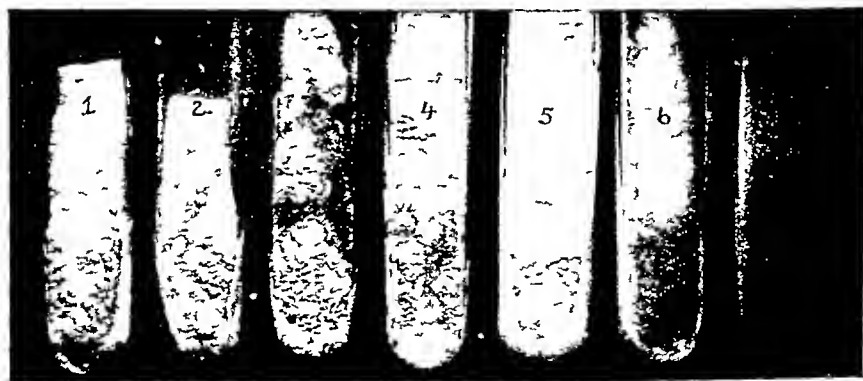


Fig 6—Growth of acid-fast Day bacilli on different mediums planted with a suspension containing 0 000 001 mg per cc and incubated four weeks at 37° C. Note growth on all the potato and egg mediums at this time and none on the glycerol agar or Long's medium. From left to right the tubes of medium are (1) glycerol water potato cylinder medium (2) glycerol water crystal violet potato cylinder medium (3) medium P (25 per cent ground potato 2½ per cent glycerol 1½ per cent agar) (4) Petroff's gentian violet egg medium (5) Dorset's egg medium (6) Long's nonprotein agar medium and (7) 5 per cent glycerol broth agar

methods for isolating tubercle bacilli were not applicable to the isolation of smegma bacilli and since there existed no certain and reliable method for the primary isolation of this microorganism and investigations concerning the smegma bacilli usually had to be confined to the use of old laboratory strains of uncertain heritage. To learn more about the reactions to chemicals and the

nutrient requirements of the more common nonpathogenic acid fast bacilli and especially as these may differ from the pathogenic tubercle bacilli was the purpose of the following studies

A Growth of Nonpathogenic Acid Fast Bacilli on Various Mediums—In order to determine the nutrient efficiency of the different mediums graded suspensions of the various nonpathogenic acid fast bacilli were carefully prepared, and with these suspensions, the different mediums were planted and were then incubated at 37° C and the amount of growth recorded at weekly intervals. The mediums tested were those used in our earlier studies with



Fig 7—Growth of acid fast timothy bacilli on different mediums planted with a suspension containing 0.000 001 mg per cc and incubated four weeks at 37° C. Note growth on all the potato and egg mediums at this time and none on the glycerol agar or Long's medium. From left to right the tubes of medium are (1) glycerol water potato cylinder medium (2) glycerol water crystal violet potato cylinder medium (3) medium 'P' (25 per cent ground potato 2½ per cent glycerol 1½ per cent agar) (4) Petroff's gentian violet egg medium (5) Dorset's egg medium (6) Long's nonprotein agar medium and (7) 5 per cent glycerol broth agar.



Fig 8—Reaction of smegma bacilli to various chemical reagents as determined by their growth after five weeks at 37° C on glycerol water crystal violet potato cylinder medium following treatment of a suspension containing 0.000 1 mg per cc with an equal volume of the reagent (designated in the photograph) for thirty minutes at 37° C and diluting with sterile saline solution the sediment after centrifugation being planted. Note the growth on the control potato medium tube and on the tube which received the bacilli treated with the acetic acid reagent but not with the other reagents.

tubercle bacilli and included Long's nonprotein agar medium⁵ containing 2½ per cent glycerol, 5 per cent glycerol broth agar medium, Dorset's egg medium,⁶ Petroff's gentian violet egg medium,⁷ a 6 per cent glycerol water plain potato cylinder medium, 6 per cent glycerol water crystal violet potato cylinder medium,⁸ and a ground potato glycerol agar medium containing 25 per cent by weight of ground autoclaved potato 2½ per cent glycerol and 1½ per cent agar (the latter labeled Medium 'P' in Tables III to X).

The acid-fast bacilli used in this study included a smegma bacillus (Hygienic Laboratory strain), an avirulent avian bacillus (obtained from Dr Esmond R Long of the University of Chicago), a virulent avian bacillus (obtained from Dr Wm H Feldman of the Mayo Foundation), a grass bacillus (from Dr Long), two rapidly growing acid-fast bacilli "K" and "Day" (obtained from Dr A A Day of Northwestern University), a butter bacillus (from Dr Long) and a timothy bacillus (obtained from Dr S A Petroff of Trudeau Sanatorium). All of these bacilli except the virulent avian strain from Dr Feldman have been maintained in our laboratory for from at least two to five years. The graded seedings with suspensions of the different bacilli on the different mediums were made in gradations of one to ten dilutions usually from amounts of 1 mg per cc to 0 000,000,001 mg per cc, each complete experiment with one strain of bacilli being run in duplicate and each dilution of seeding being tested by using four tubes of medium thus making duplicate experiments with quadruplicate tubes. The tabulations are recorded for simplicity as "+," indicating a definite positive growth of the bacilli on all the tubes planted, "?" indicating that not all the tubes were regularly positive or that one experiment proved positive and the duplicate negative, while "0" indicated that growth was absent from all the tubes planted in both experiments. The culture tubes were read at weekly intervals for a period of twelve weeks at incubator temperature (37° C). In these experiments no particular significance to the speed of growth was noted except in so far that most of the culture tubes planted with the heavier suspensions in the particular case under consideration revealed growth within two to three weeks with these rapid growers while with the relatively smaller seedings growth occurred even only after four weeks incubation. In Table III is recorded the findings obtained with the rapidly growing acid-fast smegma bacillus (Hygienic Laboratory strain).

TABLE III

GROWTH OF SMEGMA BACILLI (HYGIENIC LABORATORY) ON DIFFERENT MEDIUMS PLANTED WITH SUSPENSIONS CONTAINING GRADED AMOUNTS OF BACILLI

MEDIUM USED FOR GROWTH	AMOUNT OF BACILLI IN MG IN SUSPENSION USED FOR SEEDING THE CULTURE TUBES					
	0 01	0 001	0 000,1	0 000,01	0 000,001	0 000,000,1
Glycerol water potato cylinder medium	+	+	?	?	0	0
Glycerol water crystal violet potato cylinder medium	+	+	+	?	0	0
Medium "P" (25 per cent ground potato, 25 per cent glycerol and 15 per cent agar)	+	+	+	+	0	0
Dorset's egg medium	+	+	+	+	0	0
Petroff's gentian violet egg medium	+	+	+	+	0	0
Long's nonprotein agar medium	+	?	?	0	0	0
5 per cent glycerol broth agar medium	+	+	+	?	0	0

*The results recorded in growth of bacilli are given as the average of two experiments four tubes being seeded for each medium and each dilution of bacilli used in each experiment. + indicating all the tubes revealed growth within twelve weeks incubation. ? indicating that some of the tubes or one experiment proved negative and 0 that all the tubes were negative.

The smegma bacilli Hygiene Laboratory strain used (results recorded in Table III) grew well and virulence as low as 0.0001 mg per cc on Medium P consisting of an elevated ground potato glycerol and agar. Doves egg medium and Petroff's germin water egg medium while on glycerol agar and the potato cylinder medium, certain growth occurred only with heavier seedings 0.001 mg or heavier seedings only and as a matter of fact Long's medium proved to be a suitable requirement medium for smegma bacilli heavier than 0.001 mg per cc.



Fig. 4—Bacilli of smegma strain Hygiene Laboratory strain used (results recorded in Table III) grew well and virulence as low as 0.0001 mg per cc on Medium P consisting of an elevated ground potato glycerol and agar. Doves egg medium and Petroff's germin water egg medium while on glycerol agar and the potato cylinder medium, certain growth occurred only with heavier seedings 0.001 mg or heavier seedings only and as a matter of fact Long's medium proved to be a suitable requirement medium for smegma bacilli heavier than 0.001 mg per cc.



Fig. 5—Pearl's avirulent avian tubercle bacilli to varying chemical reasons as determined by their growth at 37°C on glycerol water cylinder medium. The first tube on the left shows a dense, dark, uniform growth throughout the liquid. The second tube shows a similar but slightly less dense growth. The third tube shows a very light, sparse growth. The fourth tube shows almost no visible growth. The fifth tube shows a moderate, uniform growth throughout the liquid. The sixth tube on the right shows a dense, dark, uniform growth throughout the liquid.

The avirulent avian acid fast bacilli grew on these medium with smaller seedings than did the smegma bacilli, as is to be noted from the data recorded in Table IV.

The glycerol water potato cylinder medium and Medium P proved best suited for supporting the growth of the avirulent avian tubercle bacilli, a seeding with suspension containing 0.0000001 mg per cc still give positive

tive growths on this medium Long's medium proved least suitable for the growth of these bacilli (growing only when seeded with suspensions containing 0 000,01 mg per c c) while the glycerol water crystal violet potato cylinder medium, Dorset's medium, Petroff's medium and glycerol agar occupied an intermediate position

Contrasting with all the strains of rapidly growing acid-fast bacilli reported on in this study the virulent avian tubercle bacilli isolated by Dr

TABLE IV

THE GROWTH OF AVIRULENT AVIAN ACID FAST BACILLI ON DIFFERENT MEDIA PLANTED WITH GRADED SUSPENSIONS

MEDIUM USED FOR GROWTH	AMOUNT OF BACILLI IN MG IN SUSPENSION USED FOR SEEDING THE CULTURE TUBES				
	0 000,1	0 000,01	0 000,001	0 000,000,1	0 000,000,01
Glycerol water potato cylinder medium	+	+	+	+	0
Glycerol water crystal violet potato cylinder medium	+	+	+	+	0
Medium "P" (25 per cent potato, 25 per cent glycerol and 15 per cent agar)	+	+	+	+	0
Dorset's egg medium	+	+	+	?	0
Petroff's gentian violet egg medium	+	+	+	?	0
Long's nonprotein agar medium	+	+	0	0	0
5 per cent glycerol broth agar medium	+	+	+	?	0

*The markings are identical to those used in Table III and the description is given in the footnote of Table III

TABLE V

THE GROWTH OF VIRULENT AVIAN TUBERCLE BACILLI (FELDMAN) ON DIFFERENT MEDIA PLANTED WITH GRADED SUSPENSIONS

MEDIUM USED FOR CULTURE	AMOUNT OF BACILLI IN MG IN SUSPENSION USED FOR SEEDING THE CULTURE TUBES								
	1 0	0 1	0 01	0 001	0 000,1	0 000,01	0 000,001	0 000,000,1	0 000,000,01
Glycerol water potato cylinder medium	+	+	+	+	+	+	+	?	0
Glycerol water crystal violet potato cylinder medium	+	+	+	+	+	+	+	?	0
Medium "P" (25 per cent potato, 25 per cent glycerol and 15 per cent agar)	+	+	+	+	+	+	+	?	0
Dorset's egg medium	+	+	+	+	+	?	?	0	0
Petroff's gentian violet egg medium	+	+	+	+	+	+	?	0	0
Long's nonprotein agar medium	+	?	?	0	0	0	0	0	0
5 per cent glycerol broth agar medium	+	+	?	?	0	0	0	0	0

*The markings are identical to those used in Table III and the description is given in the footnote of Table III

Wm H Feldman,* and proved highly pathogenic for chickens in recent tests by Dr Henry Sewall, revealed a striking favorable growth promoting action of all the potato mediums for these bacilli as is noted from the findings recorded in Table V

It is to be noted from the data recorded in Table V that the virulent avian tubercle bacilli (Feldman) will grow on all the potato mediums when planted with suspensions containing as little as 0.000 000,1 mg of bacilli

TABLE VI

THE GROWTH OF ACID-FAST GRASS BACILLI ON DIFFERENT MEDIUMS PLANTED WITH GRADED SUSPENSIONS

MEDIUM USED FOR CULTURE	AMOUNT OF BACILLI IN MG IN SUSPENSION USED FOR SEED IN THE CULTURE TUBES				
	0.000 1	0.000 01	0.000 001	0.000 000 1	0.000 000 01
Glycerol water potato cylinder medium	+	+	+	?	0
Glycerol water crystal violet potato cylinder medium	+	+	+	?	0
Medium "P" (25 per cent potato, 25 per cent glycerol and 15 per cent agar)	+	+	+	?	0
Dorset's egg medium	+	+	+	?	0
Petroff's gentian violet egg medium	+	+	+	?	0
Long's nonprotein agar medium	+	?	?	0	0
5 per cent glycerol broth agar medium	+	?	?	0	0

The markings are identical to those used in Table III and the description is given in the footnote of Table III

TABLE VII

THE GROWTH OF ACID-FAST NON-PATHOGENIC K⁺ BACILLI ON DIFFERENT MEDIUMS PLANTED WITH GRADED SUSPENSIONS

MEDIUM USED FOR CULTURE	AMOUNT OF BACILLI IN MG IN SUSPENSION USED FOR SEED IN THE CULTURE TUBES				
	0.000 1	0.000 01	0.000 001	0.000 000 1	0.000 000 01
Glycerol water potato cylinder medium	+	+	+	?	0
Glycerol water crystal violet potato cylinder medium	+	+	+	?	0
Medium "P" (25 per cent potato, 25 per cent glycerol and 15 per cent agar)	+	+	+	?	0
Dorset's egg medium	+	+	?	0	0
Petroff's gentian violet egg medium	+	+	?	0	0
Long's nonprotein agar medium	+	?	?	0	0
5 per cent glycerol broth agar medium	+	+	?	0	0

The markings are identical to those used in Table III and the description is given in the footnote of Table III

while the egg mediums require a seeding with 0.000 001 mg suspensions and glycerol agar and Long's medium about 0.01 mg suspensions. These findings closely parallel those with virulent human and bovine tubercle bacilli previously

Note Dr Wm H Feldman isolated this strain and a large number of other avian strains from pathologic material by means of the new sulphuric acid crystal violet potato cylinder method for isolating tubercle bacilli recently described by us.

ously recorded by us⁸ and explains the success achieved by Dr. Feldman in isolating avian tubercle bacilli from pathologic materials by the use of the sulphuric acid crystal violet potato cylinder method.

Similar experiments concerned with the growth of the grass bacilli, the "K" bacilli, the butter bacilli and the Day bacilli on the various mediums are recorded in Tables VI to IX.

The acid-fast rapidly growing grass bacilli (see Table VI) grows with equal facility on the potato and egg mediums tested in that growth was still obtained with suspensions containing as low as 0 000,000,1 mg bacilli per c.c. while on Long's nonprotein medium and glycerol agar growth occurred only with slightly heavier seedings (about 0 000,001 mg per c.c. suspensions).

TABLE VIII

THE GROWTH OF ACID FAST "BUTTER" BACILLI ON DIFFERENT MEDIUMS PLANTED WITH GRADED SUSPENSIONS

MEDIUM USED FOR CULTURE	AMOUNT OF BACILLI IN MG IN SUSPENSION USED FOR SEEDING CULTURE TUBES			
	0 000,01	0 000,001	0 000,000,1	0 000,000,01
Glycerol water potato cylinder medium	+	+	?	0
Glycerol water crystal violet potato cylinder medium	+	+	?	0
Medium "P" (25 per cent potato, 25 per cent glycerol and 15 per cent agar)	+	+	?	0
Dorset's egg medium	+	+	?	0
Petroff's gentian violet egg medium	+	+	?	0
Long's nonprotein agar medium	+	?	0	0
5 per cent glycerol broth agar medium	+	?	0	0

*The markings are identical to those used in Table III and the description is given in the footnote of Table III.

The acid-fast nonpathogenic "K" bacillus (see Table VII) grew with equal facility on all the potato mediums (with suspensions of 0 000 000,1 mg), less so on the egg mediums (with suspensions of 0 000,001 mg) and about equally so on Long's medium and 5 per cent glycerol broth agar. Although

TABLE IX

THE GROWTH OF ACID FAST NONPATHOGENIC "DAY" BACILLI ON DIFFERENT MEDIUMS PLANTED WITH GRADED SUSPENSIONS

MEDIUM USED FOR CULTURE	AMOUNT OF BACILLI IN MG IN SUSPENSION USED FOR SEEDING THE CULTURE TUBES				
	0 000,1	0 000,01	0 000,001	0 000,000,1	0 000,000,01
Glycerol water potato cylinder medium	+	+	+	?	0
Glycerol water crystal violet potato cylinder medium	+	+	+	?	0
Medium "P" (25 per cent potato, 25 per cent glycerol and 15 per cent agar)	+	+	+	?	0
Dorset's egg medium	+	+	+	?	0
Petroff's gentian violet egg medium	+	+	+	?	0
Long's nonprotein agar medium	+	+	?	0	0
5 per cent glycerol broth agar medium	+	?	0	0	0

*The markings are identical to those used in Table III and the description is given in the footnote of Table III.

there is a certain parallelism with the growth of the grass bacillus, the egg mediums proved slightly less suitable for this organism ("K") than the potato mediums and the glycerol broth agar and Long's nonprotein medium about equal to the egg mediums

The growth of the acid fast butter bacillus tested on the potato and egg mediums proved to be about the same in that suspensions containing 0 000 000,1 mg per cc still were able to grow on these mediums. The ability of these bacilli to draw upon a wide and limited nutrient source is indicated by their ability to grow on Long's medium and glycerol broth agar in almost as low seedings as upon the potato and egg mediums which is in striking contrast to the slow growing pathogenic avian, bovine and human tubercle bacilli. There is also a striking parallelism between the growth of the butter bacillus and the grass bacillus (Table VI)

The growth of the acid fast nonpathogenic Dry bacilli resembled that of the rapidly growing acid fast grass and butter bacilli on the potato and egg mediums in that growth occurred with the 0 000 000,1 mg suspensions. Long's medium proved a little less suitable and with this organism glycerol broth agar proved least suitable

TABLE V

THE GROWTH OF ACID FAST TIMOTHY BACILLI ON DIFFERENT MEDIUMS PLANTED WITH GRADED SUSPENSIONS

MEDIUM USED FOR CULTURE	AMOUNT OF BACILLI IN MG IN SUSPENSION USED FOR SEEDING THE CULTURE TUBES				
	0 000 1	0 000 01	0 000 001	0 000 000 1	0 000 000 01
Glycerol water potato cylinder medium	+	+	+	?	0
Glycerol water crystal violet potato cylinder medium	+	+	+	?	0
Medium 'P' (25 per cent potato 25 per cent glycerol and 15 per cent agar)	+	+	+	?	0
Dorset's egg medium	+	+	+	?	0
Petroff's gentian violet egg medium	+	+	+	?	0
Long's nonprotein agar medium	+	?	0	0	0
5 per cent glycerol broth agar medium	+	+	?	0	0

The markings are identical to those used in Table III and the description is given in the footnote of Table III

The acid fast timothy bacilli grew with equal facility on all the potato and egg mediums in suspensions as dilute as 0 000 000,1 mg per cc thus resembling the other rapidly growing nonpathogenic acid fast bacilli studied. Long's medium proved less serviceable than glycerol broth agar for supporting the growth of the timothy bacillus

In summarizing the part of this study concerned with the growth of the rapidly growing acid fast nonpathogenic bacilli it is to be noted that practically all of these rapidly growing acid fast nonpathogens studied grew well on either potato or egg mediums in some cases slightly better on the potato mediums than on the egg mediums but in no case was a better growth noted on the egg mediums than on the potato mediums. The limit of growth on the

potato and egg mediums was with seedings of suspensions containing about 0 000,000,1 mg of bacilli (a few drops of such suspensions being used for seeding) In the case of the smegma bacilli (Hygienic Laboratory Strain) heavier seedings were required even on the most suitable medium, a suspension containing 0 000,01 mg per c c being required The strain of virulent avian tubercle bacilli grew decidedly better on the potato mediums than on the other mediums including egg mediums in this respect resembling the virulent human and bovine strains of tubercle bacilli A wide range of variability in growth of the nonpathogenic acid-fast bacilli on Long's nonprotein medium and glycerol broth agar was found but in no case was the contrast



Fig 11—Reaction of acid-fast grass bacilli to various chemical reagents as determined by their growth after three weeks at 37° C on glycerol water crystal violet potato cylinder medium following treatment of a suspension containing 0 000 1 mg per c c with an equal volume of the reagent (designated in the photograph) for thirty minutes at 37° C and diluting with sterile saline solution the sediment after centrifugation being planted Note the growth of the control potato medium tube and on the tube planted with the bacilli treated with the acetic acid reagent but not with the sulphuric acid or sodium hydroxide reagents



Fig 12—Reaction of acid-fast 'K' bacilli to various chemical reagents as determined by their growth after seven weeks at 37° C on glycerol water crystal violet potato cylinder medium following treatment of a suspension containing 0 01 mg per c c with an equal volume of the reagent (designated in the photograph) for thirty minutes at 37° C and diluting with sterile saline solution the sediment after centrifugation being planted Note the growth with this suspension at this time on the control tube and with those treated with the acetic, sulphuric and oxalic reagents but not with the hydrochloric acid and sodium hydroxide reagents

with the potato and egg mediums as striking as with the pathogenic human, bovine and avian bacilli in which case relatively heavy seedings were required to obtain growth on Long's medium and glycerol agar

B Relative Resistance of Nonpathogenic Acid-Fast Bacilli to the Action of Certain Chemical Reagents—In order to extend the observations previously recorded^{1, 3} on the effect of various chemical reagents on the pathogenic human and bovine tubercle bacilli the following experiments were performed

with the rapidly growing nonpathogenic acid fast bacilli. In addition they were also extended to include the effect on a virulent strain of avian tubercle bacilli for comparison. The reagents used included 6 per cent sulphuric acid, 5 per cent oxalic acid, 3 per cent acetic acid, 2 per cent sodium hydroxide, and 3 per cent hydrochloric acid. The reagents were added in equal volume (1 c.c.) to fine suspensions of the bacilli in sterile 0.9 per cent sodium chloride solution containing from 1.0 to 0.000,001 mg. per c.c. The mixture of reagent and bacillary suspension was shaken and placed in an incubator for thirty



Fig. 13—Reaction of acid fast butter bacilli to various chemical reagents as determined by their growth after four weeks at 37° C. on glycerol water crystal violet potato cylinder medium following treatment of a suspension containing 0.01 mg. per c.c. with an equal volume of the reagent (designated in the photograph) for thirty minutes at 37° C. and diluting with sterile saline solution the sediment after centrifugation being planted. Note especially the striking contrast between the lack of toxicity of the acetic acid reagent to these bacilli as compared to other reagents.



Fig. 14—Reaction of acid fast timothy bacilli to various chemical reagents as determined by their growth after four weeks at 37° C. on glycerol water crystal violet potato cylinder medium following treatment of a suspension containing 0.0001 mg. per c.c. with an equal volume of the reagent (designated in the photograph) for thirty minutes at 37° C. and diluting with sterile saline solution the sediment after centrifugation being planted. Note especially the striking contrast between the lack of toxicity of the acetic acid reagent to these bacilli as compared to the other reagents.

minutes at 37° C. after which it was diluted with sterile saline solution centrifuged and the sediment planted on glycerol water crystal violet potato cylinder medium. Weekly records of growth were made. The concentration of the reagents used was determined by previous experiences in the study of the effect of these reagents upon human and bovine tubercle bacilli the concentrations being chosen which were innocuous to tubercle bacilli and

which were still capable of destroying the majority of the contaminating microorganisms usually present in tuberculous sputums. An exception, however, was included in using 3 per cent acetic acid which in an earlier part of this paper was shown to have a deleterious influence upon human tubercle bacilli. It might be noted here also that the bactericidal action of all these reagents toward the contaminating microorganisms present in tuberculous sputums was practically the same for all the reagents when used in the concentrations (by weight) specified in the tabulations.

TABLE XI

REACTION OF SMEGMA BACILLI (HYGIENIC LABORATORY STRAIN) TO VARIOUS CHEMICAL REAGENTS

REAGENT USED IN EQUAL VOLUME ADDED TO SUSPENSION OF BACILLI	AMOUNT OF BACILLI IN MG PER CC OF SUSPENSION USED FOR TESTING			
	1 0	0 01	0 000,1	0 000,001
Control	4*	3	2	1
6 per cent Sulphuric Acid	2	†	0	0
5 per cent Oxalic Acid	2	†	0	0
3 per cent Acetic Acid	3	1	†	†
3 per cent Hydrochloric Acid	0	0	0	0
2 per cent Sodium Hydroxide	0	0	0	0

*The readings in growth on the glycerol water crystal violet potato cylinder medium is graded from 0 = no appreciable macroscopic growth to 4 = a good profuse growth and the readings are an average of four tubes of glycerol water crystal violet potato cylinder medium planted and in duplicate experiment observed for ten weeks at 37° C.

† ? indicates that in the dilution specified only part of the tubes revealed growth within ten weeks incubation period.

TABLE XII

REACTION OF AVIRULENT ACID FAST AVIAN BACILLI TO VARIOUS CHEMICAL REAGENTS

REAGENT USED IN EQUAL VOLUME ADDED TO SUSPENSION OF BACILLI	AMOUNT OF BACILLI IN MG PER CC OF SUSPENSION USED FOR TESTING			
	1 0	0 01	0 000,1	0 000,001
Control	4*	3	1	†
6 per cent Sulphuric Acid	2	1	0	0
5 per cent Oxalic Acid	2	†	0	0
3 per cent Acetic Acid	3	†	†	0
3 per cent Hydrochloric Acid	†	0	0	0
2 per cent Sodium Hydroxide	†	0	0	0

*The readings are graded from 0 = no macroscopic growth to 4 = a profuse heavy growth occurring on the glycerol water crystal violet potato cylinder medium.

† ? indicates that in the dilution specified only part of the tubes revealed growth within ten weeks incubation period.

The same nonpathogenic acid-fast bacilli that were included in the study with the culture mediums reported upon earlier in this paper were tested in the following studies: they included the smegma bacillus (Hygienic Laboratory Strain), the avirulent acid-fast avian bacillus, the virulent avian tubercle bacillus (Feldman), the acid-fast grass bacillus, the "K" bacillus, the acid-fast butter bacillus, the acid-fast Dav bacillus, and the acid-fast timothy bacillus. The results of these studies are recorded in Tables XI to XVIII.

It is to be noted from the study recorded in Table XI that the smegma bacillus is most resistant to the 3 per cent acetic acid while it is least resistant to 2 per cent sodium hydroxide and 3 per cent hydrochloric acid, with the 6 per cent sulphuric and 5 per cent oxalic acids lying in an intermediate position. The reaction of the smegma bacilli to the acetic acid is especially

interesting since this acid proved relatively highly toxic to human tubercle bacilli, and if the smegma bacillus (Hygienic Laboratory Strain) is representative of the various strains existing, the possibility suggests itself that this reagent may prove serviceable for the isolation of this microorganism from human sources

There is to be noted a striking parallelism between the reaction of the avirulent avian bacilli (Table XII) and the smegma bacilli (Table XI) both in reaction to the chemical reagents tested and in growth behavior (Tables III and IV) on the various mediums

TABLE XIII

REACTION OF VIRULENT AVIAN TUBERCLE BACILLI TO VARIOUS CHEMICAL REAGENTS

REAGENT USED IN EQUAL VOLUME ADDED TO SUSPENSION OF BACILLI	AMOUNT OF BACILLI IN MG PER C.C. OF SUSPENSION USED FOR TESTING			
	10	0.01	0.0001	0.000001
Control	4	3	2	1
6 per cent Sulphuric Acid	4	3	2	1
5 per cent Oxalic Acid	4	3	2	1
3 per cent Acetic Acid	4	3	2	1
3 per cent Hydrochloric Acid	4	3	2	†
2 per cent Sodium Hydroxide	4	3	2	†

*The readings are graded from 0 = no macroscopic growth to 4 = a profuse heavy growth occurring on the glycerol water crystal violet potato cylinder medium

† ? Indicates that in the dilution specified only part of the tubes revealed growth within ten weeks incubation period

TABLE XIV

REACTION OF GRASS BACILLI TO VARIOUS CHEMICAL REAGENTS

REAGENT USED IN EQUAL VOLUME ADDED TO SUSPENSION OF BACILLI	AMOUNT OF BACILLI IN MG PER C.C. OF SUSPENSION USED FOR TESTING			
	10	0.01	0.0001	0.000001
Control	4*	3	2	1
6 per cent Sulphuric Acid	2	†	0	0
5 per cent Oxalic Acid	1	†	0	0
3 per cent Acetic Acid	4	3	2	1
3 per cent Hydrochloric Acid	1	0	0	0
2 per cent Sodium Hydroxide	1	0	0	0

The readings are graded from 0 = no macroscopic growth to 4 = a profuse heavy growth occurring on the glycerol water crystal violet potato cylinder medium

† ? Indicates that in the dilution specified only part of the tubes revealed growth within ten weeks incubation period

In contrast to the reaction of the avirulent acid fast avian bacillus (Table XII) to the chemical reagents tested the virulent strain of avian tubercle bacilli reveals a decided resistance to the action to all the reagents indicating that any one of these reagents may be used for isolating these bacilli although the preference would seem to be for the sulphuric oxalic or acetic acids as compared to the hydrochloric acid or sodium hydroxide. It is noteworthy also that the 3 per cent acetic acid proved innocuous in contrast to its action on human tubercle bacilli and in agreement with the effect upon the avirulent acid fast avian bacilli and the smegma bacilli. This fact, if borne out with other avian strains suggests a means of differentiating avian from human and bovine tubercle bacilli. This is now being further studied by us and will be reported on more fully at a later date

The strain of grass bacilli tested (Table XIV) revealed a high resistance to the 3 per cent acetic acid reagent and a decided bactericidal effect of all the other reagents tested, thus showing a certain parallelism to the reaction of the *Smegma* bacilli (Table XI) and the avirulent acid-fast avian bacilli (Table XII)

TABLE XV

REACTION OF ACID FAST "K" BACILLI TO VARIOUS CHEMICAL REAGENTS

REAGENT USED IN EQUAL VOLUME ADDED TO SUSPENSION OF BACILLI	AMOUNT OF BACILLI IN MG PER CC OF SUSPENSION USED FOR TESTING			
	1 0	0 01	0 000,1	0 000,001
Control	4*	3	2	††
6 per cent Sulphuric Acid	3	1	0	0
5 per cent Oxalic Acid	2	†	0	0
3 per cent Acetic Acid	3	2	0	0
3 per cent Hydrochloric Acid	†	0	0	0
2 per cent Sodium Hydroxide	0	0	0	0

*The readings are graded from 0 = no macroscopic growth to 4 = a profuse heavy growth occurring on the glycerol water crystal violet potato cylinder medium

† † indicates that in the dilution specified only part of the tubes revealed growth within ten weeks incubation period

TABLE XVI

REACTION OF ACID FAST BUTTER BACILLI TO VARIOUS CHEMICAL REAGENTS

REAGENT USED IN EQUAL VOLUME ADDED TO SUSPENSION OF BACILLI	AMOUNT OF BACILLI IN MG PER CC OF SUSPENSION USED FOR TESTING			
	1 0	0 01	0 000,1	0 000,001
Control	4*	3	2	1
6 per cent Sulphuric Acid	2	1	††	0
5 per cent Oxalic Acid	1	†	0	0
3 per cent Acetic Acid	3	2	2	†
3 per cent Hydrochloric Acid	1	0	0	0
2 per cent Sodium Hydroxide	1	0	0	0

*The readings are graded from 0 = no macroscopic growth to 4 = a profuse heavy growth occurring on the glycerol water crystal violet potato cylinder medium

† † indicates that in the dilution specified only part of the tubes revealed growth within ten weeks incubation period

TABLE XVII

REACTION OF ACID FAST "DAY" BACILLI TO VARIOUS CHEMICAL REAGENTS

REAGENT USED IN EQUAL VOLUME ADDED TO SUSPENSION OF BACILLI	AMOUNT OF BACILLI IN MG PER CC OF SUSPENSION USED FOR TESTING			
	1 0	0 01	0 000,1	0 000,001
Control	4*	3	2	1
6 per cent Sulphuric Acid	3	2	1	0
5 per cent Oxalic Acid	3	2	1	0
3 per cent Acetic Acid	4	3	2	1
3 per cent Hydrochloric Acid	0	0	0	0
2 per cent Sodium Hydroxide	0	0	0	0

*The readings are graded from 0 = no macroscopic growth to 4 = a profuse heavy growth occurring on the glycerol water crystal violet potato cylinder medium

The acid-fast "K" bacillus revealed a striking susceptibility to all the reagents tested, it being more pronounced with hydrochloric acid and sodium hydroxide. Even the 3 per cent acetic acid reagent which displayed a lack of toxicity to the majority of the acid-fast nonpathogenic bacilli also displayed a distinct toxicity to the "K" bacillus.

It is to be noted from the results recorded in Table XVI that there is a striking resemblance between the reaction of the acid fast butter bacilli to that of the acid fast grass bacilli (Table XIV)

The reaction of the acid fast Day bacilli would appear different from some of the other rapid growers tested in that in addition to slight toxicity of the sulphuric acid and oxalic acid reagents the acetic acid reagent proved entirely innocuous, while both the hydrochloric acid and sodium hydroxide reagents displayed a profound effect upon these bacilli, thus differing from the rapidly growing "K" and butter bacilli

TABLE XVIII

THE REACTION OF ACID-FAST TIMOTHY BACILLI TO VARIOUS CHEMICAL REAGENTS

REAGENT USED IN EQUAL VOLUME ADDED TO SUSPENSION OF BACILLI	AMOUNT OF BACILLI IN MG PER C C OF SUSPENSION USED FOR TESTING			
	10	0.01	0.0001	0.000001
Control	4	3	2	1
6 per cent Sulphuric Acid	2	1	0	0
5 per cent Oxalic Acid	2	1	0	0
3 per cent Acetic Acid	4	3	2	1
3 per cent Hydrochloric Acid	0	0	0	0
2 per cent Sodium Hydroxide	1	0	0	0

The readings are graded from 0 = no macroscopic growth to 4 = a profuse heavy growth occurring on the glycerol water crystal violet potato cylinder medium

There is a distinct resemblance of the reaction of the acid fast timothy bacillus (Table XVIII) to the various reagents tested and that of the smegma bacillus (Table XI), the acid fast grass bacillus (Table XIV), the acid fast butter bacilli (Table XVI), and the Day bacilli (Table XVII) in that the acetic acid reagent is more innocuous to these bacilli than the sulphuric acid or oxalic acid reagents, while the hydrochloric acid and the sodium hydroxide reagents proved decidedly toxic to all these strains

In summarizing the results of these studies on the reactions to various chemical reagents of the rapidly growing acid fast bacilli as compared to the virulent avian tubercle bacilli and the pathogenic human and bovine tubercle bacilli it is to be noted particularly that there is a striking resemblance in the reaction of all the nonpathogens in that they display a decided resistance to the action of the 3 per cent acetic acid reagent and a pronounced susceptibility to the 3 per cent hydrochloric acid reagent, and the 2 per cent sodium hydroxide reagent. The reaction of these bacilli to the 6 per cent sulphuric acid reagent and the 5 per cent oxalic acid reagent lies intermediate to the effect of the acetic acid and the hydrochloric acid or sodium hydroxide reagents. In the case of the grass, butter, Day and timothy bacilli the 3 per cent acetic acid proved so innocuous that these bacilli even in high dilutions grew about as well as the untreated controls on the glycerol water crystal violet potato cylinder medium. It was interesting to note that the virulent strain of avian tubercle bacilli tested proved highly resistant to the action of all the chemical reagents tested which suggested the possibility of utilizing this fact for differentiating these bacilli culturally from the human and bovine tubercle bacilli especially if this fact is borne out in further tests with other virulent strains of avian tubercle bacilli. The reaction of the rapidly growing

acid-fast bacilli to the sulphuric and oxalic acid reagents also offered an explanation for the fact that only the pathogens in contaminated tuberculous materials would survive this treatment, and thus account for the reliability of the sulphuric or oxalic acid crystal violet potato cylinder method for diagnostic purposes as well as elucidating the fact that this procedure proved impractical for the isolation of smegma bacilli in our hands. On the basis of these observations it seems likely that the 3 per cent acetic acid reagent may prove serviceable for isolating smegma bacilli from human sources and work is now in progress to test this out.*

SUMMARY AND CONCLUSIONS

1 In comparative tests a 5 per cent oxalic acid reagent proved superior to a 6 per cent sulphuric acid reagent for isolating tubercle bacilli from tuberculous sputums in that a greater percentage of the total tubes planted yielded positive cultures of tubercle bacilli which was accounted for by the lesser toxicity of the oxalic reagent as determined by bacteriostatic tests and also due to a greater germicidal action of this reagent on contaminating organisms. The oxalic acid reagent also possessed advantages over the sulphuric acid as a reagent inherent in being a solid easily weighed, stable and obtainable in pure crystalline form.

2 A study of the quantitative growth characteristics of a group of non-pathogenic acid-fast bacilli including a strain of smegma, grass, butter, timothy, "K," "Day" and an avirulent avian bacilli on various nutrient mediums used for growing tubercle bacilli revealed that these bacilli grow better on potato and egg mediums as compared to glycerol broth agar and a non-protein agar medium (Long's) especially when the culture tubes are planted with small numbers of bacilli. The same proved true of a virulent avian tubercle bacillus studied. Potato or egg mediums are therefore suggested as most suited to the isolation of these bacilli when present in small numbers. With heavy seedings they grew about equally well on all the mediums tested.

3 In a study of the reaction of the nonpathogenic acid-fast bacilli to various chemical reagents, it was found that a 3 per cent acetic acid reagent added in equal volume to different amounts of fine suspensions of these bacilli proved comparatively innocuous while capable of efficiently destroying the usual contaminating microorganisms in tuberculous sputums, suggesting that this acid should prove serviceable for the isolation of this class of microorganisms and particularly the smegma bacillus.

The lack of toxicity of the 3 per cent acetic acid reagent to the rapidly growing acid-fast bacilli and a strain of virulent avian tubercle bacilli is in striking contrast to its toxic effect upon virulent human and bovine tubercle bacilli, and if borne out with more extensive tests with strains of virulent avian tubercle bacilli suggests the use of this reagent for differentiating this microorganism culturally from the other two pathogenic varieties.

A 3 per cent hydrochloric acid reagent and a 2 per cent sodium hydroxide reagent proved highly toxic to all the nonpathogenic acid-fast bacilli tested,

*The authors are grateful to Mr L. D. Miller for his kind assistance in pursuing the technical details of this study.

while a 6 per cent sulphuric acid reagent and a 5 per cent oxalic acid reagent usually occupied an intermediate position

In contrast to both the pathogenic human and bovine tubercle bacilli which were highly susceptible to the action of the acetic acid reagent and resistant to the sulphuric and oxalic acid reagents and the nonpathogenic acid fast bacilli that displayed a decided resistance to the acetic acid reagent and were sensitive to the sulphuric acid and oxalic acid reagents, the virulent strain of avian tubercle bacilli tested proved resistant to all the acid reagents tested as well as the 2 per cent sodium hydroxide reagent

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IMPROVED COLORIMETRIC PROCEDURES FOR THE QUANTITATIVE ESTIMATION OF THE PROTEINS OF THE CEREBRO-SPINAL FLUID*

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ORIGIN OF PROTEINS

FREMONT-SMITH and Ayer¹ maintain that the origin of the proteins normally present in the cerebrospinal fluid is largely a matter of conjecture. The ventricular fluid contains much less protein than the lumbar fluid. These observers state that it is possible that the protein of normal ventricular fluid comes through the choroid plexus from the blood plasma, or that it is a product of the ependymal cells. It probably represents the minimal portion of the perivascular fluid which enters the ventricles. The amount of proteins in the normal ventricular fluid is very slight.

Fremont-Smith and Ayer hold that the increasing amount of protein found in the lumbar fluid may be due to the larger number of perivascular spaces emptying into the subarachnoid space. Other possible sources of the proteins may be the arachnoid cells, the lymph spaces within the nerve roots, and the central canal of the cord.

These observers maintain that, in view of the uncertainty as to the origin of the proteins normally found in the cerebrospinal fluid, it is impossible to come to a definite conclusion in regard to the source or sources of the proteins found in pathologic states. All of the possible sources mentioned for normal proteins and in addition transudation through dilated meningeal vessels may be the sources of the proteins in disease.

The relative proportions of albumin and globulin as found in the blood and the relative increase of globulin over albumin in the blood in disease would support the theory that a certain portion of the protein of the cerebrospinal fluid has its origin in the blood.

Ayer and Foster² hold that protein excess of the cerebrospinal fluid is an index not only of an exudative meningeal process, but also of the degree of permeability of meningeal vessels under pathologic or abnormal physiologic states. In the one case the protein is of exudative, in the other of transudative origin. The sources of origin are therefore diverse.

Hewitt³ is of the opinion that a mere leakage phenomenon does not altogether account for the presence of the increased amount of proteins, since albumin with its smaller molecular size would probably diffuse more readily

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and thus the albumin globulin ratio would increase and not decrease, as occurs in certain forms of neurosyphilis

Robertson⁴ holds that normally the globulin of the blood serum is less abundant than the albumin fraction. In animals or human beings infected with the streptococcus or staphylococcus, however, the ratio may be much more than unity.

The question of the cause of this change in the blood serum is of importance to our understanding of the mechanisms by which the organism protects itself against infections. In diphtheria, for instance, the antitoxins are found in the globulin fraction of the serum.

The cause of the increase of the globulin of the serum in infections is still unknown, although a number of observers have presented experimental theories which appear reasonable.

Buck, quoted by Robertson,⁴ has shown that if ether or chloroform be administered for very prolonged periods to animals so that albuminuria begins to appear, the globulin quotient rises far more markedly than could be accounted for by an escape of serum albumin into the urine. This indicates that possibly the true source of the marked alterations in the globulin quotient, which occur in infections, is the result of an increased permeability of the tissue cells.

It appears highly probable that one of the principal sources of the globulin of cerebrospinal fluid is the blood. If this is so, then the factors which are responsible for an increase of the globulin of the blood are also responsible for an increase of the globulin of the cerebrospinal fluid.

NATURE OF PROTEINS

Hewitt³ found that the proteins in normal cerebrospinal fluid consist of albumin and globulin, the albumin being as a general rule in excess of the globulin, the greater portion of the globulin being pseudoglobulin. Euglobulin may also be found in normal cerebrospinal fluid and is usually increased in amount in diseased conditions. In pathologic states, both albumin and globulin are increased, the globulin being increased more in proportion than is albumin.

Thus Hewitt found that the ratio of albumin to globulin in normal spinal fluid was 8 to 1, in tabes 3 to 1, and in paresis about 1.3 to 1. This observer noted that the smallness of the albumin to the globulin ratio follows the strength of the Wassermann reaction and the extent of the precipitation of the colloidal gold solution.

Lange assumed that in syphilitic and parietic affections specific proteins occurred in the cerebrospinal fluid, and these abnormal proteins caused the various colloidal gold precipitation curves.

Felton, quoted by Hewitt,³ is of the opinion that abnormal relative amounts of globulin and albumin in the fluid are responsible for the colloidal gold reaction.

Weston, Cruickshank, Mellanby and Anwyl Davies⁵ held that globulin occurred in syphilitic and parietic affections in abnormally large amounts. These findings were not, however, the result of accurate quantitative analyses.

Hewitt³ found that the total protein increase alone was not the chief factor in paretic fluid, and that the precipitation of colloidal gold solution must be attributed to other conditions because of the fact that the cerebrospinal fluid of meningitis and other conditions have a high total protein content, but these do not give the characteristic precipitation of gold solution.

Greenfield and Carmichael maintain that there is no pathologic human cerebrospinal fluid in which the amount of globulin exceeds that of albumin. According to these observers, the proportion of albumin to globulin in various diseases is as follows: acute meningitis and arteriosclerosis 12 to 1, paresis 7 to 3, spinal tumor 2 to 1.

Greenfield and Carmichael further state that of the three globulins, pseudoglobulin is found in normal fluid. In pathologic conditions euglobulin and fibrinogen make their appearance. These two globulins are found in alterations of the cerebrospinal fluid and are associated with the presence of various antibodies. For instance, the Wassermann reacting substances, as well as hemolysin, separate on dialysis along with the englobulin fraction. Some of the precipitins may be found in the pseudoglobulin fraction. Complement is usually found in the fibrinogen fraction.

STATE OF PROTEINS IN SPINAL FLUID

Kafka⁶ holds that the various proteins in cerebrospinal fluid are subject to the same physicochemical changes as are the proteins of the blood serum.

Robertson,⁴ in speaking of the proteins of the blood serum, states that there is much reason for believing that they are not merely present therein as a mixture, but in the form of a chemical complex possessing a different physical and chemical property from those of the constituent proteins out of which it is built up. It is felt that this same property applies to the total protein complex as well as to the integral protein fractions of cerebrospinal fluid.

Fremont-Smith and Aver¹ maintain that it is possible that the protein present in normal ventricular fluid comes through the choroid plexus from the blood plasma. In addition, transudation through dilated meningeal vessels may be the source of the proteins. There is therefore reason for the opinion that the various proteins of the cerebrospinal fluid have physical and chemical properties similar to those of the proteins of the blood.

PROTEIN CONTENT VALUES OF SPINAL FLUID

Aver and Foster² observed that as the result of quantitative protein examinations of spinal fluid, some of the values which at first were believed to be normal were finally considered definite increases. They also noted that by means of quantitation the border-line between normal and pathologic amounts of protein is more closely drawn.

These observers found that by means of quantitative estimations of the total protein of cerebrospinal fluid, the progress of treatment could be gauged in a manner similar to the study of the cell count, the Wassermann test, or the colloidal gold test. In fact, they noted that the total protein content of the cerebrospinal fluid began to fall early as the result of treatment in most diseases of the nervous system, except in paresis.

Aver and Foster, in their investigation, found that the amount of protein

in normal spinal fluid lies between 16 mg and 38 mg per 100 c.c., and that more than 40 mg is suspicious of an underlying diseased condition

Fremont Smith and Aver¹ observed that in many border line cases the total protein of cerebrospinal fluid may be more than doubled and yet the globulin test is negative. This indicates the need of exact methods for the estimation of albumin and globulin of the cerebrospinal fluid. They found that the amount of protein normally present in the lumbar fluid varied between 15 mg and 45 mg per 100 c.c.

Many observers have found that in the treatment of paresis the total protein may remain elevated after all other laboratory tests of the cerebrospinal fluid have become normal.

Mestrezat considered 15 mg to 30 mg of total protein as normal while values over 35 mg were considered pathologic. Esliuchen considered 20 mg to 30 mg as normal.

Some observers have found that in many fluids in which the protein content is normal the Wassermann test is positive. This may be explained by the fact that the Wassermann reacting substances are present in euglobulin and this fraction may be increased without there being an increase of the total protein content of the fluid.

Hewitt² found that 30 mg per 100 c.c. was the normal quantity of total protein. This observer found that the normal albumin content was about 20 mg per 100 c.c. Globulin was present to the extent of 3 mg per 100 c.c. Euglobulin was found to the extent of 0.5 mg per 100 c.c.

QUANTITATIVE DETERMINATION OF PROTEINS OF SPINAL FLUID

Aver² in collaboration with Denis elaborated a method for the estimation of total protein which has proved fairly satisfactory and not too difficult of application. This method consists of precipitating the cerebrospinal fluid proteins in a colloidal state by means of sulphosalic acid and reading the turbidity in the unknown by means of a colorimeter against a standard solution prepared at the same time from a blood serum of known content.

According to this technique the amount of protein in normal spinal fluid lies between 16 mg and 38 mg per 100 c.c. More than 40 mg is considered suspicious of an underlying pathologic process.

Young and Bennett³ have devised a method which consists of precipitating the total protein of cerebrospinal fluid by alcohol acetic acid and heat, then measuring the resultant precipitate volumetrically in capillary tipped vaccine tubes and reading it in terms of milligrams per 100 c.c. According to this method, normal readings of total protein vary from 25 mg to 75 mg per 100 c.c.

H. Wu⁴ made use of the color reaction of proteins of the blood with phosphomolybdic tungstic acid (phenol reagent). The proteins of the blood serum were precipitated and separated from each other. Phenol reagent was then added to bring about a color reaction the color being compared with a standard tyrosine solution. The color of the protein obtained upon the addition of the phenol reagent is due to its tyrosine content and is constant for any given protein.

In 1926, Ling⁹ applied Wu's colorimetric method for the estimation of proteins of blood serum to spinal fluid and determined the total protein of a small number of specimens of fluid. No attempt was made by Ling to determine the separate protein fractions.

Hewitt³ elaborated colorimetric methods for the determination of the total protein, and the various protein fractions of cerebrospinal fluid, based upon the method of Wu with blood serum.

The method consists in precipitating the globulin from the fluid by the addition of an appropriate amount of ammonium sulphate and the determination of the protein content of the original fluid and in the filtrates from the ammonium sulphate precipitation. This is done by precipitation of the proteins by tungstic acid and measuring the color produced by the precipitates with phenol reagent.

MODIFIED PROCEDURES FOR THE DETERMINATION OF SPINAL FLUID PROTEINS

The methods for the determination of albumin, globulin and total protein of the cerebrospinal fluid described herein are based upon the work of Hewitt³ and Wu⁵. According to our procedure, individual techniques are used for the quantitative estimation of globulin and total protein. The albumin content is obtained by deducting the globulin from the amount of total protein.

Wu⁵ found the tyrosine equivalents of globulin and albumin to be as follows

1 mg of tyrosine equals $\begin{cases} 25.2 \text{ mg globulin} \\ 27.5 \text{ mg albumin} \end{cases}$

Reagents used in modified procedure

- 1 Tyrosine solution
- 2 Standard solution of tyrosine (weak)
- 3 Standard solution of tyrosine (strong)
- 4 Phenol reagent
- 5 20 per cent sodium carbonate
- 6 1 per cent sodium hydrate

TYROSINE SOLUTION

Dissolve 50 mg tyrosine (Pfaendstiel) in 250 cc of N/10 HCl. This solution usually does not deteriorate after standing for a period of six to eight months.

WEAK AND STRONG TYROSINE STANDARDS

It has been found that, in order to make colorimetric comparisons, two standards should be prepared simultaneously with the unknown. A weak standard containing 0.1 mg tyrosine and a strong standard containing 0.2 mg tyrosine. The particular standard to be used in the determination depends upon which of the two standards the color of the unknown solution approximates.

The tyrosine standard solutions are made up as follows:

- 1 Into one of two 25 cc glass stoppered cylinders place 1 cc tyrosine solution containing 0.2 mg tyrosine (strong standard), into the other, place 0.5 cc tyrosine solution containing 0.1 mg tyrosine (weak standard).
- 2 Add to each cylinder 0.25 cc phenol reagent and distilled water up to 20 cc.
- 3 Add 2.5 cc 20 per cent sodium carbonate.
- 4 Add distilled water up to 25 cc.

PHENOL REAGENT

1 Into a clean 1000 cc pyrex beaker, place 100 gm sodium tungstate, 20 gm phosphomolybdic acid, 50 cc phosphoric acid (85 per cent) and 750 cc distilled water.

- 2 Boil mixture continuously for two hours
- 3 Cool at room temperature and dilute with distilled water to 1000 cc Filter if necessary The final product should be of a brilliant canary yellow color

TECHNIC FOR GLOBULIN DETERMINATION*

- 1 Place 5 cc cerebrospinal fluid in a 15 cc centrifuge tube and add 5 cc chemically pure saturated solution ammonium sulphate
- 2 Place tube in water bath at 56 °C until maximum precipitation occurs This usually takes about thirty minutes
- 3 Centrifuge at high speed for about ten minutes until the globulin precipitate becomes firmly packed at bottom of tube Pour off supernatant fluid completely and drain the tube thoroughly by inverting Remove any of the ammonium sulphate at the mouth of the tube with filter paper
- 4 Add 1 cc of 10 per cent sodium tungstate and shake bottom of tube until the globulin precipitate is dissolved
- 5 Remove this alkaline globulin solution with a pipette into another clean 15 cc centrifuge tube, taking care that the pipette does not touch the walls of the tube and that no ammonium sulphate is carried over
- 6 Add 4 cc distilled water and 1 cc 2/3 N sulphuric acid The globulin is thus again precipitated
- 7 Centrifuge for five minutes and discard the supernatant fluid
- 8 Add 2 or 3 drops of 1 per cent sodium hydroxide and shake bottom of tube to help dissolve globulin precipitate
- 9 Add 2 cc distilled water and 0.1 cc phenol reagent
- 10 Add 1 cc 20 per cent sodium carbonate
- 11 Add distilled water to a volume of 10 cc and set tube aside for fifteen minutes for maximum blue color to develop
- 12 Compare clear, blue solution with weak or strong tyrosine standard prepared simultaneously

GENERAL FORMULA FOR GLOBULIN OR TOTAL PROTEIN CALCULATION

$$\frac{S}{U} \times SS \times \frac{V_u}{V_s} \times \frac{100}{X} = \text{mg per 100 cc cerebrospinal fluid}$$

Explanation of formula

$$\frac{S}{U} = \frac{\text{Reading of standard}}{\text{Reading of unknown}}$$

Note The fraction $\frac{S}{U}$ varies depending upon the reading of the standard and the reading of the unknown Ordinarily the standard is set at 20 The denominator of the fraction $\frac{S}{U}$ indicates the reading of the unknown which is variable

SS = Strength of standard (10 mg per cc)

Note The SS factor varies with the type of standard used If the strong standard is used SS equals 0.2 mg If the weak standard is used SS equals 0.1 mg

$$\frac{V_u}{V_s} = \frac{\text{Volume of unknown}}{\text{Volume of standard}}$$

Note This fraction is constant The unknown in each instance being brought up to a volume of 10 cc and the standard being brought up to a volume of 25 cc Therefore $\frac{V_u}{V_s} = \frac{10}{25}$

$$\frac{100}{X} = \frac{\text{Per 100 cc cerebrospinal fluid}}{\text{Amount of cerebrospinal fluid used in the determination}}$$

Note The denominator of the fraction $\frac{100}{X}$ varies depending upon the amount of cerebrospinal fluid used in the determination

If unknown solution is turbid centrifugate and transfer supernatant fluid to colorimeter cup

APPLICATION OF GENERAL FORMULA TO ESTIMATE QUANTITY OF GLOBULIN

Five cc cerebrospinal fluid was used in the determination of globulin. The strong standard was used, the colorimeter was set at 20, and the reading of the unknown was 16, therefore

$$\frac{20}{16} \times 0.2 \times \frac{10}{25} \times \frac{100}{5} = 2 \text{ mg tyrosine globulin per 100 cc}$$

Since 1 mg tyrosine equals 25.2 mg globulin, then

$$25.2 \times 2 = 50.4 \text{ mg globulin per 100 cc}$$

TOTAL PROTEIN DETERMINATION

1 Place 2 cc cerebrospinal fluid in a 15 cc graduated centrifuge tube, add 1 cc 10 per cent sodium tungstate and 1 cc 2/3 N sulphuric acid. Mix thoroughly and set aside at room temperature for thirty minutes, or until maximum precipitation of the proteins takes place.

2 Centrifuge at high speed for about 10 minutes.

3 Pour off supernatant fluid.

4 Add 2 or 3 drops of 1 per cent sodium hydroxide and shake bottom of tube.

5 Add 3 cc distilled water and 0.1 cc phenol reagent.

6 Add 1 cc 20 per cent sodium carbonate solution.

7 Add distilled water to a volume of 10 cc and allow to stand for fifteen minutes, until maximum blue color develops.

8 Compare the unknown with weak or strong standard as in globulin determination.

APPLICATION OF GENERAL FORMULA TO ESTIMATE QUANTITY OF TYROSINE TOTAL PROTEIN

Two cc cerebrospinal fluid was used in the determination of total protein. The strong standard was used, the colorimeter was set at 20, and the reading of the unknown was 15, therefore

$$\frac{20}{15} \times 0.2 \times \frac{10}{25} \times \frac{100}{2} = 5.33 \text{ mg tyrosine total protein per 100 cc}$$

DETERMINATION AND COMPUTATION OF ALBUMIN

In order to estimate the amount of albumin in cerebrospinal fluid, it is necessary to subtract the tyrosine globulin from the tyrosine total protein.

In the foregoing example it is as follows:

$$5.33 - 2 = 3.33 \text{ mg tyrosine albumin per 100 cc}$$

Since the tyrosine equivalent for albumin is 27.5, then

$$27.5 \times 3.33 = 91.6 \text{ mg albumin per 100 cc}$$

MANNER OF ESTIMATING AMOUNT OF TOTAL PROTEIN

In order to estimate the amount of total protein it is necessary to add the albumin and globulin, therefore

$$91.6 + 50.4 = 142 \text{ mg total protein per 100 cc}$$

TECHNICAL DIFFICULTIES

In using this method, turbid solutions are frequently obtained, the cause of which is either chemical or technical. Generally, the turbidity is due to the presence of sodium salts, chiefly carbonates, and is the result of the addition of 20 per cent sodium carbonate, which is used to dissolve the protein precipitate. In the technique described herein, 2 or 3 drops of 1 per cent sodium hydroxide are used initially in dissolving the protein precipitate, this tends to eliminate turbidity.

Another cause of turbidity in the unknown solution is the incomplete removal of ammonium sulphate used to precipitate the globulins. It is therefore important to remove this salt as completely as possible so that the final solution is of a clear blue color.

Both the unknown and the standard solutions should be prepared simultaneously and allowed to stand for the same length of time, so that the maximum development of blue color will take place in both solutions.

GENERAL CONSIDERATIONS

During the conduct of this investigation it was our aim to ascertain the following points:

1 The total protein, globulin and albumin of normal cerebrospinal fluid, using this modified procedure, also the variations in such diseases as paresis, tabes, tertiary syphilis, multiple sclerosis, meningitis, etc.

2 The ratio of albumin to globulin in normal spinal fluid, as well as in pathologic fluids.

3 The relationship of the cerebrospinal fluid proteins to the colloidal gold test.

4 The effect of treatment upon the individual protein fractions of cerebrospinal fluid, as well as upon the total protein.

THE PROTEINS IN NONMENINGITIC AND NONSYPHILITIC CEREBROSPINAL FLUIDS

On account of the inability to obtain cerebrospinal fluid from strictly normal subjects, it was necessary to use specimens from subjects under treatment for various miscellaneous conditions which are ordinarily not accompanied by abnormal protein contents.

Table I shows that, in a series of 10 nonsyphilitic and nonmeningitic cerebrospinal fluids, the albumin content ranged from 21 mg to 52 mg per 100 c.c., the average being 34 mg.

In the same series the globulin content ranged from 6 mg to 20 mg per 100 c.c., the average being 13 mg.

The total protein ranged from 27 mg to 62 mg per 100 c.c. the average being 48 mg.

The ratio of albumin to globulin in the 10 cases studied ranged from a high of 5.2:1 to a low of 1.4:1. The average ratio of albumin to globulin being 2.6:1.

TABLE I

PROTEINS OF CEREBROSPINAL FLUID IN NONMENINGITIC AND NONSYPHILITIC DISEASES

NUMBER	MG PER 100 CC			RATIO OF ALBUMIN TO GLOBULIN	COLLOIDAL GOLD CURVE	DIAGNOSIS
	ALBUMIN	GLOBULIN	TOTAL PROTEIN			
1	25	18	43	1.4:1	0001100000	Aortic aneurysm
2	30	15	45	2.0:1	0011000000	Aortic insufficiency
3	45	13	58	3.5:1	0011100000	Chronic alcoholism
4	42	12	54	3.5:1	0011100000	Dementia precox
5	26	12	38	2.2:1	1112221000	Influenza
6	40	12	52	3.3:1	1112210000	Constitutional psychopathic state
7	30	14	44	2.1:1	0011000000	Dementia precox
8	33	20	53	1.7:1	0011210000	Hypomania
9	52	10	62	5.2:1	1112210000	Manic depressive psychosis
10	21	6	27	3.5:1	1112210000	Hysteria

PROTEINS OF CEREBROSPINAL FLUID IN UNTREATED CASES OF PARESIS

In reviewing the data of Table II it is noted that in the examination of the proteins of 16 specimens of cerebrospinal fluid from cases of paresis, the albumin content ranged from 35 mg to 160 mg per 100 c c, the average being 65 mg

The globulin content ranged from 21 mg to 93 mg per 100 c c, the average being 44 mg

The total protein content ranged from 63 mg to 204 mg per 100 c c, the average being 110 mg

The ratio of albumin to globulin varied from a high of 3.6:1 to a low of 0.6:1, the average being 1.5:1

In normal cerebrospinal fluid the amount of albumin invariably exceeds the amount of globulin. It is noted that in the untreated cases of paresis reported in Table II the amount of globulin is almost equal to that of albumin, in fact, in three of the specimens reported in Table II the quantity of globulin exceeded the quantity of albumin (Cases 1, 4, and 16)

TABLE II
CEREBROSPINAL FLUID PROTEINS IN UNTREATED CASES OF PARESIS

NUMBER	MG PER 100 C C			RATIO OF ALBUMIN TO GLOBULIN	COLLOIDAL GOLD CURVE
	ALBUMIN	GLOBULIN	TOTAL PROTEIN		
1	68	93	161	0.7:1	555555331
2	82	33	115	2.5:1	5555532100
3	49	32	81	1.5:1	5555552100
4	42	69	111	0.6:1	5555543210
5	42	21	63	2.0:1	5555544310
6	58	27	85	2.1:1	5555443100
7	82	69	151	1.2:1	1224333210
8	67	25	92	2.7:1	5555553210
9	84	45	129	1.9:1	555555331
10	35	30	65	1.2:1	5555432100
11	57	47	104	1.2:1	5555553210
12	55	45	100	1.2:1	5555554431
13	60	43	103	1.4:1	5555553210
14	59	28	87	2.1:1	5555553210
15	160	44	204	3.6:1	5555554210
16	44	60	104	0.7:1	5555534210

PROTEINS OF CEREBROSPINAL FLUID IN TABES DORSALIS

The quantitative estimation of the total protein, as well as of the albumin and globulin contents of cerebrospinal fluid in 20 specimens of fluid from cases of tabes shows that the albumin content ranged from 23 mg to 63 mg per 100 c c, the average being 42 mg

The globulin content ranged from 8 mg to 46 mg per 100 c c, the average being 20 mg

The total protein content ranged from 39 mg to 87 mg per 100 c c, the average being 62 mg

The ratio of albumin to globulin varied from a high of 6.8:1 to a low of 0.5:1, the average being 2.1:1

TABLE III
CEREBROSPINAL FLUID PROTEINS IN TABES DORSALIS

NUMBER	MG PER 100 C.C			RATIO OF ALBUMIN TO GLOBULIN	COLLOIDAL GOLD CURVE
	ALBUMIN	GLOBULIN	TOTAL PROTEIN		
1	40	14	63	3 5 1	0011100000
2	49	14	63	3 5 1	0011100000
3	54	8	62	6 8 1	0011100000
4	23	26	49	0 0 1	1122100000
5	53	13	66	4 1 1	1122221000
6	52	15	67	3 5 1	1122°10000
7	63	16	79	4 0 1	1122332100
8	43	0	5	4 8 1	1112221000
9	25	46	71	0 5 1	1223332100
10	23	31	54	0 7 1	1234432100
11	41	16	57	2 6 1	1122100000
12	50	12	62	4 2 1	1233321000
13	48	18	66	2 7 1	1112221000
14	38	15	53	2 5 1	1112210000
15	39	35	74	1 1 1	1123321000
16	31	26	57	1 2 1	1123321000
17	47	40	87	1 2 1	1234432100
18	60	15	75	4 0 1	1223321000
19	24	15	30	1 6 1	0011110000
20	28	16	44	1 8 1	0011100000

PROTEINS OF CEREBROSPINAL FLUID IN TERTIARY SYPHILIS

Table IV lists the results of 31 determinations of cerebrospinal fluid of cases of tertiary syphilis. It is noted that the albumin content ranged from 25 mg to 79 mg per 100 c.c., the average being 45 mg.

The globulin ranged from 8 mg to 25 mg per 100 c.c., the average being 16 mg.

The total protein ranged from 37 mg to 104 mg per 100 c.c., the average being 61 mg.

The ratio of albumin to globulin varied from a high of 7.6:1 to a low of 1.4:1, the average being 2.9:1.

It is noted that the quantities of the various proteins of cerebrospinal fluid of cases of tertiary syphilis are in excess of those in nonmeningitic and nonsyphilitic conditions. Whether or not tertiary syphilis is accompanied by an involvement of the central nervous system with a concomitant increase of the proteins of the cerebrospinal fluid cannot be definitely stated at this time, however, the data in Table IV would lead one to infer that this is a possibility.

PROTEINS OF CEREBROSPINAL FLUID IN MULTIPLE SCLEROSIS

In the course of this study estimations were made of the albumin, globulin and total protein contents of cerebrospinal fluid of a number of cases of multiple sclerosis, with a view of ascertaining whether or not the quantities of these fractions were comparable with those of parietic cerebrospinal fluid, the object being to determine the reason for the similarity of the colloidal gold curves in these two conditions.

Table V reveals the fact that the albumin content varied from 43 mg to 117 mg per 100 c.c., the average being 71 mg. In paresis, the range was from 35 mg to 160 mg per 100 c.c., the average being 65 mg.

TABLE IV
CEREBROSPINAL FLUID PROTEINS IN TERTIARY SYPHILIS

NUMBER	MG PER 100 CC			RATIO OF ALBUMIN TO GLOBULIN	COLLOIDAL GOLD CURVE
	ALBUMIN	GLOBULIN	TOTAL PROTEIN		
1	43	22	65	2 0 1	1122210000
2	59	12	71	4 9 1	0012310000
3	35	25	60	1 4 1	1122210000
4	40	11	51	3 6 1	1233321000
5	32	10	42	3 2 1	0122100000
6	41	20	61	2 1 1	1233321000
7	48	22	70	2 2 1	1123321000
8	42	16	58	2 6 1	1123310000
9	58	18	76	3 2 1	0011210000
10	29	19	48	1 5 1	0001100000
11	49	20	69	2 5 1	1112221000
12	35	12	47	2 9 1	1122100000
13	60	15	75	4 0 1	1122333210
14	28	13	41	2 2 1	1123321000
15	59	15	74	3 9 1	1123332100
16	36	8	44	4 5 1	1122221000
17	45	13	58	3 5 1	0011100000
18	37	19	56	1 9 1	1123321000
19	31	14	45	2 2 1	1122210000
20	39	15	54	2 6 1	1122100000
21	74	22	96	3 4 1	1122321000
22	34	20	54	1 7 1	2233321000
23	40	16	56	2 5 1	1122210000
24	79	25	104	3 2 1	2233331000
25	25	12	37	2 1 1	0011100000
26	76	10	86	7 6 1	1122210000
27	38	11	49	3 5 1	1122100000
28	47	14	61	3 4 1	0012221000
29	44	13	57	3 4 1	0012100000
30	55	12	67	4 6 1	1112221000
31	52	11	63	4 7 1	1112221000

The globulin content varied from 20 mg to 49 mg per 100 cc, the average being 33 mg. In paresis the globulin range was from 21 mg to 93 mg per 100 cc, the average being 44 mg.

The total protein content varied from 66 mg to 140 mg per 100 cc, the average being 104 mg. In paresis the range was from 63 mg to 204 mg per 100 cc, the average being 110 mg.

The ratio of albumin to globulin varied from 5 3 1 to 1 4 1, the average being 2 2 1. In paresis the ratio varied from 3 6 1 to 0 6 1, the average being 1 5 1.

The above findings indicate that there is a slight similarity of the quantities of the protein fractions in paresis and multiple sclerosis. Whether or not this accounts for the type of colloidal gold reaction in these two conditions cannot be definitely stated, inasmuch as the nature and the mechanism of this reaction are not thoroughly understood.

THE PROTEINS OF CEREBROSPINAL FLUID DURING THE TREATMENT OF PARESIS

For the purpose of ascertaining whether or not antisyphilitic treatment has any effect on the albumin, globulin, and total protein contents, determinations were made on a series of cases while receiving treatment.

TABLE V
CEREBROSPINAL FLUID PROTEINS IN MULTIPLE SCLEROSIS

CASE NUMBER	DATE	MG PER 100 CC			RATIO OF ALBUMIN TO GLOBULIN
		ALBUMIN	GLOBULIN	TOTAL PROTEIN	
1	10/27/27	91	49	140	1.9 1
	5/21/28	117	22	139	5.3 1
	7/13/28	~	43	115	1.7 1
2	7/13/28	43	23	66	1.9 1
3	7/13/28	48	20	68	2.4 1
4	7/13/28	56	39	95	1.4 1

The findings may be seen by referring to Table VI. The albumin, globulin and total protein of cerebrospinal fluid of 21 cases of paresis were studied and it was found that the albumin content was reduced in 17 or 81 per cent of the cases, the globulin content was reduced in 13 or 62 per cent and the total protein was reduced in 17, or 81 per cent of the cases during the treatment regime.

It is interesting to note that during this period the ratio of albumin to globulin was reduced in 12 or 57 per cent of the cases, an increase of the ratio was observed in 8 cases, and in 1 case no change was noted.

The above may be explained by the fact that treatment caused a reduction of the albumin content of spinal fluid more often and more readily than the globulin content, with the result that the ratio of albumin to globulin was reduced more frequently than it was increased.

THE AVERAGE PROTEIN CONSTITUENTS IN VARIOUS DISEASES

In reviewing Table VII it is noted that the smallest quantities of the various proteins are to be found in the nonmeningitic and nonsyphilitic diseases.

A slight increase of the proteins was found in the cerebrospinal fluid of cases of tertiary syphilis.

The largest quantities of the various proteins are found in paresis and multiple sclerosis.

The ratio of albumin to globulin is high in the nonmeningitic and nonsyphilitic fluids. The lowest ratio of albumin to globulin was found in cerebrospinal fluids of paresis.

THE RELATIONSHIP OF THE CEREBROSPINAL FLUID PROTEINS TO THE COLLOIDAL GOLD REACTION

One of the purposes of this study was to ascertain whether or not changes of the protein quantities or changes in the quantitative relation of albumin to globulin were in any way responsible for the various characteristic colloidal gold curves.

J. Cruickshank¹⁰ has observed that the globulin obtained from normal spinal fluid, even when used in concentrated form, is almost inactive when added to a solution of colloidal gold. He therefore is of the opinion that the precipitating action of parietic fluid cannot be ascribed to this protein fraction alone, but is probably dependent on a specific alteration of the physical state

TABLE VI
PROTEINS OF CEREBROSPINAL FLUID IN PARESIS DURING TREATMENT

CASE NUMBER	DATE	MG PER 100 CC			RATIO OF ALBUMIN TO GLOBULIN	COLLOIDAL GOLD CURVE	TREATMENT
		ALBUMIN	GLOBULIN	TOTAL PROTEIN			
1	10/26/27	55	15	70	3 7 1	5555553200	Tryparsamide
	1/ 9/28	57	27	84	2 1 1	4433321000	and
	3/21/28	68	27	95	2 5 1	1233321000	Mercury
2	10/25/27	41	32	73	1 3 1	5555544321	Tryparsamide
	1/ 5/28	72	40	112	1 8 1	5554431000	and
	3/19/28	35	17	52	2 1 1	5554443100	Mercury
3	11/ 7/27	84	49	133	1 7 1	2233321000	Tryparsamide
	2/ 2/28	102	37	139	2 8 1	2223333100	and
	4/19/28	104	22	126	4 7 1	1233544210	Mercury
4	11/23/27	56	21	77	2 7 1	1233331000	Tryparsamide
	2/ 6/28	49	20	69	2 5 1	2233321000	Mercury and
	4/30/28	35	30	65	1 2 1	1233321000	Bismuth
5	12/ 1/27	49	76	125	0 6 1	5555555210	Malaria Inocula
	1/19/28	49	44	93	1 1 1		tions, Tryparsa
	3/26/28	33	32	65	1 0 1	3555443210	mide and Mercury
6	12/ 5/27	44	27	71	1 6 1	5555532100	Tryparsamide
	2/20/28	40	22	62	1 8 1	5555543210	and
	4/23/28	35	23	58	1 5 1	2345531000	Mercury
7	1/ 3/28	66	40	106	1 7 1	1112221000	Tryparsamide
	3/15/28	61	24	85	2 5 1	3343321000	and
	5/28/28	65	26	91	2 5 1	5333441000	Mercury
8	10/26/27	82	37	119	2 2 1	5555544321	Tryparsamide
	3/19/28	63	22	85	2 9 1	5555543210	and
	5/31/28	56	19	75	2 9 1	1233331000	Mercury
9	11/ 4/27	38	15	53	2 5 1	1233310000	Tryparsamide
	5/ 5/28	41	17	58	2 4 1	1123332100	and Mercury
10	11/28/27	69	32	111	2 2 1	5555554210	Tryparsamide
	4/26/28	46	33	79	1 4 1	3444432100	and
	7/27/28	49	37	86	1 3 1	2554332100	Bismuth
11	11/28/27	47	23	70	2 0 1	5555432100	Tryparsamide
	2/ 9/28	39	19	58	2 1 1	2233210000	and Mercury
12	12/ 5/27	78	33	111	2 4 1	5555554210	Tryparsamide
	3/ 1/28	40	30	70	1 3 1	2443431000	and Mercury
13	11/ 7/27	44	29	73	1 5 1	1122331000	Tryparsamide
	4/ 2/28	29	19	48	1 5 1	2223331000	and Mercury
14	12/ 5/27	72	35	107	2 1 1	5555554210	Tryparsamide
	2/27/28	46	26	72	1 8 1	2235432100	and Mercury
15	12/ 8/27	50	21	71	2 4 1	5555543100	Sodium Iodide
	2/ 3/28	47	21	68	2 2 1	5554321000	and Mercury
16	12/13/27	36	19	55	1 9 1	5554432100	Tryparsamide
	3/ 5/28	30	17	47	1 8 1	2233321000	and Mercury
17	12/19/27	44	14	58	3 1 1	1112221000	Sulpharsphenamine
	3/ 1/28	38	20	58	1 9 1	1112221000	and Mercury
18	12/19/27	56	19	75	2 9 1	1112332100	Tryparsamide
	3/ 1/28	38	20	58	1 9 1	1122333100	and Mercury
19	3/ 8/28	48	35	83	1 4 1	5555543100	Tryparsamide
	5/21/28	37	29	66	1 3 1	5555553100	and
	8/ 1/28	29	50	79	0 6 1	5555532000	Mercury
20	1/ 5/28	51	23	74	2 2 1	2233210000	Tryparsamide
	5/31/28	38	15	53	2 5 1	2333321000	and Mercury
21	10/26/27	57	22	79	2 6 1	1233321000	Tryparsamide
	3/29/28	63	22	85	2 9 1	1123431000	and
	7/16/28	76	17	93	4 5 1	1122310000	Mercury

TABLE VII

SHOWING THE AVERAGE PROTEIN CONSTITUENTS ALSO ALBUMIN GLOBULIN RATIO IN VARIOUS DISEASES

	AVERAGE ALBUMIN	AVERAGE GLOBULIN	AVERAGE TOTAL PROTEIN	AVERAGE RATIO ALBU MIN TO GLOBULIN
Non meningitic and non syphilitic diseases	34	13	48	2.6 1
Tertiary syphilis	45	16	61	2.9 1
Tabes dorsalis	42	20	62	2.1 1
Paresis	85	44	110	1.5 1
Multiple sclerosis	71	33	104	2.2 1

of the globulin which is associated with an increased electric charge. Cruickshank further maintains that this alteration of the globulin cannot be regarded as specific for syphilis since it may occur also in multiple sclerosis. He states that the phenomenon of colloidal gold reaction is dependent upon the albumin and globulin contents of the fluid, the precipitation of the gold being due to the globulin and its retention in solution being due to the albumin fraction.

Mellanby and Anwyl Davies,¹¹ as a result of their studies, established the hypothesis that the precipitating factors of the colloidal gold solution were either euglobulin or pseudoglobulin or a combination of these. The euglobulin being the active agent which precipitates colloidal gold, and the pseudoglobulin fraction tends to keep the gold in solution and antagonizes the precipitating action of the euglobulin. These observers are of the opinion that all cerebrospinal fluids contain a constant quantity of pseudoglobulin but variable quantities of euglobulin and that the different colloidal gold reactions are due to the varying quantities of euglobulin.

If this assumption is correct, then the characteristic precipitation of the gold solution obtained in paresis and multiple sclerosis should not persist after treatment when the globulin fraction is diminished in amount and the ratio of albumin to globulin is increased.

The data in Table VI shows that the characteristic colloidal gold precipitation is not due entirely to the relative quantities of globulin and albumin in the cerebrospinal fluid because instances are seen where the globulin is decreased and albumin increased, and yet the typical parietic curve persists. Furthermore, in reviewing the findings of Case No. 1 of Table V, in which there were three separate estimations of the protein fractions the examination of October 27, 1927, in which the ratio of albumin to globulin was 1.9 1, was accompanied by a typical parietic curve (5555543210) while the examination of July 13, 1928 in which the ratio of albumin to globulin was similar (1.7 1), was accompanied by a diminished precipitation of the gold (2233310000).

Unfortunately a study was not made of the relative quantities of euglobulin and pseudoglobulin fractions of the cerebrospinal fluid in various diseases so that it is impossible to confirm the hypothesis of Mellanby and Anwyl Davies that variations of the euglobulin fraction caused the various characteristic colloidal gold curves.

It may therefore be concluded that there is some phenomenon other than the quantities of albumin and globulin or the relative proportions of albumin to globulin which is responsible for the characteristic colloidal gold precipitation reactions in the various diseases. Whether the precipitation of the colloidal gold solution is dependent upon a physical change of the globulin, i.e., upon an increased electric charge, as maintained by Cruickshank, or is dependent upon the amount of euglobulin in the fluid cannot be stated at this time.

SUMMARY AND CONCLUSIONS

1 On account of the importance of the proteins of the cerebrospinal fluid as an aid to diagnosis, as well as for the guidance of treatment, it is felt that the quantitative determination of these constituents should be done routinely.

2 Accordingly, the method of Wu for the estimation of the blood proteins and that of Hewitt for spinal fluid proteins have been modified and a colorimetric technic has been developed.

3 By this modified procedure the quantities of globulin and total protein are estimated and the albumin is determined by deducting the amount of globulin from the total protein.

4 a The average quantities per 100 c.c. of the various proteins of non-meningitic and nonsyphilitic cerebrospinal fluids were: albumin, 34 mg., globulin, 13 mg., total protein, 48 mg. The average ratio of albumin to globulin was 2.6:1.

b The average quantities per 100 c.c. of the various proteins of spinal fluid in untreated paretics were: albumin, 65 mg., globulin, 44 mg., total protein, 110 mg. The average ratio of albumin to globulin was 1.5:1.

c The average quantities per 100 c.c. of the various proteins of spinal fluid in tabes dorsalis were: albumin, 42 mg., globulin, 20 mg., total protein, 62 mg. The average ratio of albumin to globulin was 2.1:1.

d The average quantities per 100 c.c. fluid of the various proteins of cases of tertiary syphilis were: albumin, 45 mg., globulin, 16 mg., total protein, 61 mg. The average ratio of albumin to globulin was 2.9:1.

It is noted that the quantities of the various proteins of cerebrospinal fluids of tertiary syphilis without apparent involvement of the central nervous system were slightly in excess of the quantities of the proteins in nonmeningitic and nonsyphilitic fluids.

e The average quantities of the various proteins per 100 c.c. fluid in cases of multiple sclerosis were: albumin, 71 mg., globulin, 33 mg., total protein, 104 mg. The average ratio of albumin to globulin was 2.2:1.

It is noted that the quantities of the different protein fractions in this condition are similar to those of paresis. It is possible that the type of colloidal gold curve found in these two conditions is due to the similarity of the quantities of the protein fractions. Inasmuch as the quantitative estimation of the euglobulin fraction was not made it was not possible to confirm the hypothesis of Mellanby and Anwyl-Davies that variations of the euglobulin fraction cause the characteristic colloidal gold curves.

5 A study was made of the proteins of the cerebrospinal fluid of a number of cases of paresis while undergoing treatment. It was found that during treatment a reduction of the albumin fraction took place. The globulin content was not influenced as readily, nor as frequently as was the albumin. The ratio of albumin to globulin varied in more than half of the cases the ratio was decreased during treatment.

Improvement should be followed by an increase of the albumin to globulin ratio. The decrease was evidently due to the fact that the albumin fraction was more greatly influenced by treatment than the globulin fraction.

6 The smallest quantities of the various proteins were found in the non-meningitic and nonsyphilitic fluids. The largest quantities of the various proteins were found in paresis and multiple sclerosis. The ratio of albumin to globulin was high in the nonmeningitic and nonsyphilitic fluids. The lowest ratio of albumin to globulin was found in paresis.

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QUANTITATIVE MICROSCOPIC URINALYSIS*

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INTRODUCTION

IN THE course of a year I see hundreds of urinalysis reports from different laboratories throughout the United States and Canada, and it is interesting to note that all of them report their microscopic findings in terms which predicate the existence of quantitative methods of urinary microscopy. It is also noteworthy that our textbooks do not suggest that microscopic urinalysis is a quantitative procedure and that the literature touches on the subject only in connection with a few special studies which utilize modified blood counting techniques to enumerate the microscopic objects in urine.

In our work, many specimens come from people who also send their urines to other laboratories, and the examinations bring to light numerous discrepancies which the interested laboratories must account for in order to appease the disturbed minds of physicians and patients. In such instances the undeniable alibi that the urine a person passes may vary from time to time usually gets by, by virtue of its silent intimation that the reports would have agreed had both laboratories examined portions of the same urine.

It also often happens in our experience, however, that different laboratories do examine portions of the same urine and nevertheless report similar discrepancies. In such instances alibis lose their smugness and ordinarily degenerate into one laboratory testing, as it were, on its findings while what may be called the "negative" laboratory sidesteps with the implication that some laboratories find pus or casts, as the case may be, in every urine. Unfortunately, such occurrences are frequent and acutely embarrassing because they create a situation which clinicians and laymen cannot understand without resorting to theories that discredit clinical pathology and pathologists.

The thinking laboratory man, of course, knows better. To paraphrase an old saying about the spirit being willing but the flesh weak, he knows that he reports his microscopic findings in terms which are conventional rather than actually quantitative, and those who have not considered the matter may learn from a few experiments that differences in techniques do not account for all of the discrepancies because well-trained technicians who carry through a stereotyped technic in the same laboratory on portions of the same specimen of urine will report results that vary by hundreds of per cent.

Uranalyses are made so frequently and repeatedly that it seemed to me more than desirable to remove a situation which so often reproaches clinical

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pathology because of the perplexities and irritations it causes not only laboratory men and clinicians but also, and in increasing numbers nowadays, laymen

The work here outlined was therefore undertaken with the object of improving techniques so as to increase precision, because only in this way did a chance seem open to enable different laboratories to report microscopic findings with something like uniformity

For the sake of clarity the material is divided into two parts. Part I reviews the methods currently practiced by different laboratories and the experimental checks which were made of the various manipulative details entering into the techniques of urinary microscopy. Part II presents, step by step, the details of a practicable procedure for microscopic urinalysis which has experimentally and in practice proved accurate enough to enable reporting results with a uniformity satisfactory for all clinical purposes. The new procedure requires for its performance no more time and trouble than the system heretofore employed in the Prudential Laboratory.

Part I

CURRENT METHODS

To get as complete information as possible concerning the current practices of different laboratories, a questionnaire was sent to 100 laboratories which were known to specialize more or less in urinalysis and with whom we have had occasion, from time to time, to compare reports. These laboratories are scattered all over North America and are headed by men who come from different schools. The questionnaire deals with the various technical details involved in microscopic urinalysis, and I take this opportunity not only to acknowledge the 100 per cent responses but also to express my obligations for the complete and careful answers, and especially to thank those who favored me with suggestions, opinions, experiences and, in a few cases, personal demonstrations thus making the present effort practically a cooperative one.

As the problem concerned only equipment and manipulative details, the questionnaire was framed to elicit such information with the utmost simplicity and clearness.

QUESTIONNAIRE

A. Microscope

- 1 Make of microscope?
- 2 Tube length employed?
- 3 Achromatic or apochromatic system?
- 4 Maker's designation of eyepieces used?
- 5 Maker's designation of objectives used?
- 6 Combination of eyepiece and objective used for 1/6 field?

B. Sediment

- 1 Quantity of urine centrifuged?
- 2 At what speed?
- 3 For how long?
- 4 Is all or only part of sediment examined?

- 5 Please give full details of your technique for sampling sediment and preparing slide
- 6 Size of slide used?
- 7 Size of cover glass used?
- 8 Do you examine more than one slide? If so, how many?
- 9 How many fields do you cover with low power? With high power?
- 10 Have you experimented with any other methods? If so, please give details, etc

It will be seen that the first Part A of the questionnaire covers equipment. It develops the fact that over 95 per cent of the laboratories work with the achromatic systems of standard American microscopes. In this connection, it is not amiss to note that it is particularly in the examination of unstained and colorless specimens, such as urinary sediments, that the advantages of better resolution and freedom from chromatic aberration offered by apochromatic systems more than compensate for their increased cost.

The laboratories use eyepieces running all the way from 2 to 15 power but substantially conform with one another in the employment of 16 and 4 mm objectives for analysis. One or another of every combination of these lenses is in use by the laboratories for counting the objects in urinary sediments, which, of course causes discrepancies because the area of a microscopic field varies with a particular lens combination, even with a particular make, although 16 mm objectives as a rule cover an area eighteen times greater than 4 mm objectives of the same make.

For generations the textbooks on clinical pathology have recommended 4 mm objectives for urinary microscopy, and it is undoubtedly true that the 4 mm or even a higher power objective is essential for satisfactory resolving structural and cytologic details, and that every microscope used for examining urinary sediments should carry a high power objective on its nosepiece. Indispensable as it is, however, for seeing fine details, the 4 mm or higher power objective is not only unnecessary but even disadvantageous and inexpedient for counting objects which can be easily identified with lower powers or less magnification. The higher the power of an objective the smaller is the field it covers, the shorter is its working distance, the less its penetration, the more finicky its focusing, and the greater its sensitiveness to thickness of cover glass and specimen. The greater penetrations afforded by 16 mm objectives enable them to disclose objects lying in different planes which are much more likely to remain unseen and therefore uncounted when 4 mm objectives are used. Those who feel that 16 mm objectives do not give them sufficient magnification for counting would do well to try an 8 mm objective. I have found them extremely well suited for counting the objects in urinary sediments because they give magnifications intermediate between 4 and 16 mm objectives and are more comfortable to work with than 4 mm objectives.

To sum up microscopic equipment, the questionnaires indicate such wide differences in practice between the different laboratories that I am led to suggest that better uniformity in reporting can be secured by an understanding to consider some definite area or some certain fixed volume as a standard unit for counting objects in urinary sediments. Then by some simple correction

factor, each individual or laboratory with the lens equipment they prefer can nevertheless put their reports on the same footing with other laboratories. As an example the Prudential Laboratory employs apochromatic systems and counts with 8 mm objectives and 15 power compensating eyepieces. This combination covers an area between two and three times the area covered by the usual 10 power eyepiece and 4 mm achromatic objectives but our reports become comparable and harmonize with those who count with 10 power eyepieces and 4 mm achromatic objectives by the simple process of dividing our counts by $2\frac{1}{2}$. Microscope makers readily supply data concerning the areas covered by their lenses and the simplest kind of calculation will enable any individual using any equipment he prefers to report his findings uniformly with others and thus eliminate those discrepancies which are due to differences in microscopic equipment.

The second Part B of the questionnaire covers the manipulative and other details included in examining and reporting counts of red blood pus and epithelial cells and casts etc, in urinary sediments. In general the answers to the questionnaire expose a very disconcerting diversity of laboratory practice. In fact the answers indicate that it is doubtful if any two laboratories make microscopic examinations of urine in exactly the same way.

As the questionnaires were returned they were reviewed and roughly classified as to general technical design with special reference to sensitiveness and quantitative accuracy. To illustrate one laboratory examines the sediment of 120 cc of urine and another that of only 5 cc. If both laboratories received the same urine and examined the entire sediment as some do one would report 100 pus cells while the other would report about 4 perhaps none at all, because the technique of one laboratory is 25 times more sensitive than that of the other. On the other hand when 5 and 120 cc samples of the same urine were treated differently following details actually given in the questionnaires, the 5 cc sample sometimes yielded more sensitive or positive results than the 120 cc sample or 25 times more urine.

From this it is evident that the problem of defining a microscopic procedure capable of giving quantitative and therefore uniform results was only to be solved by checking, step by step the various technical procedures delineated by the answers given in the questionnaires. In order to make our study as complete as possible other techniques found in the textbooks and literature such as those of Addis¹ Ockerblad² Heitzmann³ and others were also investigated. For the sake of convenience and clarity and to guide our experiments all of the manipulative details involved in urinary microscopy were listed as follows:

SAMPLING

- Sampling the urine
- Sedimenting the urine
- Sampling the sediment (microscopic specimen)

MICROSCOPY

- Preparing the specimen
- Examining the specimen
- Reporting the findings

It cannot escape attention that each step on the list connotes the performance of several distinct technical details, also that it was obligatory to perform the same manipulations many times and in various ways in order to gain the experience and data essential for comparing with and checking against one another all of the different techniques outlined in the literature and questionnaires comprised in our program. From this it will be understood that this paper would run to an inordinate length were the attempt made to set forth fully all of the protocols accumulated in the course of the work. It is therefore necessary to limit the presentation of data to forms of summary or illustration in commenting on particular technical details.

SAMPLINGS

Sampling the Urine

If it is intended to examine the urine immediately after voiding or if the design be to sediment by gravity, no mixing is required. In all other instances the whole amount of urine voided should be well mixed before a sample is taken for examination or mailing. As a pitfall of frequent error, the importance of proper sampling cannot be overemphasized, and is experimentally demonstrable by the thousands of percentage differences in results found when different parts of the same insufficiently mixed urine are examined. No attempt to quantitate can succeed if the sample selected for examination be not true or representative. To insure thorough mixing, gently turning the bottle upside down a few times has proved as satisfactory as any other method, but it is particularly noteworthy that violent agitation is not only unnecessary but positively harmful because it tends to injure casts and otherwise damage the fragile objects we intend to examine under the microscope. It is a good plan to clear the specimen as far as possible of interfering suspended matter like urates, etc., before taking samples.

According to the questionnaires, almost all laboratories sample 15 c c, a few sample 10 c c, and a few only 5 c c. On the other hand, some laboratories sample 2 ounces, or twelve times more, and others 4 ounces, making a 25-fold variation in the sample of urine examined by different laboratories. On this point a long line of related experiments indicate that the volume of urine sampled is relatively unimportant and that no particular advantages accrue from sampling larger amounts than the usual 15 c c sampled by more than 85 per cent of the laboratories. It is the efficiency of sampling and subsequent technique of handling rather than the amount of the sample which determine the sensitiveness and quantitative accuracy of the final result.

Sedimenting the Urine

The questionnaires develop the fact that only one laboratory relies on gravity alone and that all of the others depend more or less upon the centrifuge for sedimenting urine. Some laboratories, however, allow the urine to stand for a time, sedimenting by gravity before centrifuging a sample selected from the bottom layers.

The controversy over the relative merits of sedimenting urine by gravity or centrifuge centers chiefly on statements made by some authorities in text-

books and discussions to the effect that rapid centrifugalization deforms and injures fragile objects like leucocytes and casts. The point is important but, unfortunately, does not lend itself to illustration by experimental proof. I have, however, on numerous occasions sedimented by gravity and by centrifuge (2 to 3000 r p m) urines containing not only blood pus, and epithelial cells but also urines containing more fragile objects like waxy and fatty casts and hyaline and fatty detritus and then compared the sediments under the microscope. What I have seen convinces me that centrifugal speed within such limits as I have used it does not injure nor deform any of these objects unless centrifugalization be unduly prolonged. In this case violence is done by the packing of the sediment which takes place, and insult is added to injury in the process of extricating some of the sediment for examination. Other injurious forces come into play during deceleration of the centrifuge and in pouring off the supernatant urine or part of the sediment, because during deceleration and pouring eddies form which produce torques that exert unequal forces and thus tend to injure delicate objects by twisting them. On the other hand, centrifugal force is constant steady and evenly distributed and therefore much less likely to injure delicate objects. By avoiding unnecessarily prolonged centrifuging in order to prevent packing the sediment and by decelerating smoothly, a better conditioned sediment can actually be had with the centrifuge than by gravity.

The only other advantage which preliminary sedimentation by gravity might be conjectured to offer is increased sensitiveness, and on this point the methods given in the questionnaires were checked experimentally. Thus, the most sensitive technic given in the questionnaires was described as follows: 'We plan to have a 4 ounce specimen. This is allowed to stand for at least one hour and the top three fourths or four fifths is poured off. Fifteen c c of the remainder is centrifuged for three minutes at 1050 r p m.' The efficiency of this procedure was checked by carefully performing every detail of the technic on a specimen of normal urine to which was added fresh blood from a finger prick. Blood counts showed that the specimen contained 244,800,000 red cells. After standing an hour sedimenting by gravity, the upper three fourths, or 90 c c, were poured off and 15 c c, or half of the remainder, were centrifuged three minutes at 1100 r p m. The red cells in the sediment were then counted and only 17,612,000 found, indicating a recovery of only about 60 per cent of the actual number of red cells in the 15 c c sample (hemolysis controlled). As a more sensitive criterion the red cells in the urine overlying the sediment were also counted, and both counts were found to agree within experimental error. A number of correlative experiments also indicate that the effect of an hour's preliminary sedimentation by gravity is negligible in the absence of efficient centrifugalization.

The questionnaires also reveal that some laboratories underdo and other laboratories overdo centrifuging. Thus one laboratory centrifuges only one minute while several centrifuge fifteen minutes and one or more laboratories centrifuge for each interval between these. Similarly, the different labora-

tories centrifuge urine at different speeds ranging from 500 to 3000 rpm with various rpm's in between the extremes practiced by one or more of the laboratories

No good reason for such wide variations in the performance of a routine technical detail are apparent, because, after all, the shaping of an adequate or efficient sedimenting technic is a simple matter of mathematics and mechanics which easily lends itself to experimental proof. Fourteen years ago I centrifuged urines containing casts and red and white blood cells at different rpm's for different intervals of time in order to determine the time and speed factors necessary to bring 90 per cent or more of the microscopic objects in urine down into the sediment, and as a result the Prudential Laboratory has since then centrifuged urines for four and a half minutes at 2000 rpm.⁴ This technic employs the usual 15 cc centrifuge tube and has proved efficient, and does very little if any, injury to the microscopic objects. These early experiments have been rechecked during the course of the present study, and Tables

TABLE I

VOL CC	NORMAL URINE + RBD BLOOD CELLS RED CELLS	CENTRIFUGED AT 2000 R P M MINUTES	NO CELLS FOUND IN SEDIMENT	NO CELLS FOUND IN SUPERNATANT URINE
5	19,600,000	1	CF*	200
		2	CF	80
		3	CF	44
		4	CF	36
5	196,000	1	84	16
		2	84	0
		3	96	0
		4	108	0
10	47,800,000	1	14,240	432
		2	CF	248
		3	CF	56
		4	CF	40
10	478,000	1	432	108
		2	520	68
		3	560	8
		4	960	0
15	72,900,000	1	24,400	13,600
		2	46,400	2,400
		3	CF	1,600
		4	CF	410
15	729,000	1	260	410
		2	308	100
		3	350	80
		4	510	56

*Cover the field

I and II give the results of centrifuging and counting experiments. Two different types of laboratory centrifuges with speeds controlled by tachometers were used. Counts were made with standard hemacytometers and Euscope

The experimental results were confirmed with specimens from cases of hematuria and pyuria

TABLE II

MINUTES AT 2000 R.P.M.	CELLS PER C.C. REMAINING IN SUPERNATANT URINE	PERCENTAGE OF REMOVAL
Hematuria		
0	1,360 000	—
2	160 000	89.1
3	20 000	98.5
4	—	100.0
Pyuria		
0	490,000	—
2	60 000	87.8
3	45,000	89.8
4	10,000	98.0
5	—	100.0

From these data it is evident that 15 c.c. urine samples in the usual type of centrifuge tube employed by practically all laboratories are efficiently and adequately sedimented by centrifuging four minutes at 2000 r.p.m. (As a practical matter, four and one half minutes includes bringing the centrifuge to speed.) If greater or less speed than 2000 r.p.m. be preferred, the correct time factor for that speed should be determined. It should bring 98 per cent or more of the microscopic objects down into the sediment without being prolonged enough to pack the sediment.

All of the experiments shown in the tables were carried out with standard laboratory 15 c.c. centrifuge tubes and the marked differences in the results obtained by centrifuging different quantities of urine i.e., 5, 10 and 15 c.c. will not have escaped attention. At first sight it is not surprising to find that smaller quantities sediment quicker than larger quantities but it would be a mistake to attribute the differences merely to volume of sample, because in the standard 15 c.c. centrifuge tube the samples stand at different heights or vary in length according to their quantity. Thus a 5 c.c. sample is 53 mm., a 10 c.c. sample is 80 mm. and a 15 c.c. sample 110 mm. long.

To determine the effect of length of sample on centrifuging three tubes of different bore and shape were selected. As a matter of convenience these were standard types of centrifuge tubes designed to hold different quantities of sample, i.e. a 50 c.c. tube having a bore at the mouth with a diameter of 27 mm., a 30 c.c. tube with a diameter of 26 mm. at its mouth and a 15 c.c. tube having a diameter of 14 mm. Into each of the three tubes 15 c.c. of the same urine containing red blood cells was poured and the lengths of the standing samples measured. In the 50 c.c. tube the urine sample measured 31 mm., in the 30 c.c. tube 62 mm. and in the 15 c.c. tube 95 mm. The samples were centrifuged different periods of time at 2000 r.p.m. and counts then made of the cells recovered from the supernatant urine which was centrifuged ten minutes at 3000 r.p.m.

The data strongly suggest a picture of sedimentation showing the microscopic objects lying in layers piled on top of one another and the time factor for efficient centrifuging is therefore the time it takes to bring the topmost layers of microscopic objects in the sample down into the sediment. Correla

tive experiments indicate that increasing the time factor by centrifuging at low speeds tends to irregularities in results and to injury of the microscopic objects by packing and other forces

The data also point to the possibility of improving sedimentation techniques by employing shorter centrifuge tubes

TABLE III
EFFECT OF LENGTH OF SAMPLES ON CENTRIFUGALIZATION

MINUTES CENTRIFUGED AT 2000 R.P.M.	LENGTH OF SAMPLE	CELLS IN SUPERNATANT URINE
1	31 mm	65,000
	62 mm	120,000
	95 mm	430,000
2	31 mm	0
	62 mm	90,000
	95 mm	130,000
3	31 mm	0
	62 mm	20,000
	95 mm	56,000
4	31 mm	0
	62 mm	0
	95 mm	20,000

Sampling the Sediment (Microscopic Specimen)

After sedimenting urine, the next step is to get a good and true sample of the sediment for microscopy. The literature and questionnaires offer many and various ways for performing this step, and as a criterion for checking their accuracy I adopted the reproducibility of results obtained by repeating the same procedure on portions of the same urine.

Sediment must first be extricated from the supernatant urine. Many simply pour this off while others depend upon different methods of pipetting. When checked experimentally, none of the methods gave reproducible results. In fact, none of them could be managed so as to avoid diluting the sediment with urine, which always drips back from the sides of the tubes or enters into the pipettes. Such unavoidable dilutions of sediment represent an uncontrollable and inconstant factor which spoils every other attempt at accuracy, and from specimens which are poor in mucus or binding material for the sediment, such as low gravity urines, etc., casts are lost as easily by one method as by another. Furthermore, the amount of mucoid binding material almost always determines the volume of sediment obtained by centrifuging. It varies greatly in both normal and pathologic urines and is, therefore, a serious factor in destroying accuracy. The specimens from Messrs X and Y illustrate this effect because both contain the same number of casts. X's urine is poor in binding material and yields only 0.1 c.c. of sediment, while Y's urine is richer in mucoid material and when similarly centrifuged yields 0.5 c.c., or 5 times more sediment. When both sediments are examined under the microscope and casts are counted with equal efficiency, the reports must show that X's urine contains 5 times as many casts as Y's. Such differences in sediment

concentration, of course, account for errors in reporting because specimens like X's are reported as having persistent casts while those like Y's are reported negative when both urines actually contain exactly the same number of casts. The mucoid material present in urines has thus the same disastrous effects on accuracy as the unavoidable dilution which occurs in extricating sediment from supernatant urine.

These serious sources of error can be minimized in fact avoided by scratch marking the centrifuge tubes at some definite level so as to define some arbitrary volume of sediment or sediment plus urine which can always be taken as a constant sediment volume. To determine a practicable volume for such a constant 100 consecutive urines having a specific gravity of 1020 or higher were centrifuged for four and a half minutes at 2000 r.p.m. with the results shown in Table IV.

TABLE IV

NO OF SPECIMENS	VOLUME OF SEDIMENT
15	Less than 0.1 cc
19	0.2 cc
32	0.3 cc
31	0.4 cc
2	0.5 cc
1	0.6 cc

Evidently 0.5 cc sediment or sediment plus urine is a practicable volume constant for 15 cc urine samples. This represents a concentration factor of 30. For the rare specimens which yield more than 0.5 cc of sediment it is easy to take 1 cc sediment or sediment plus urine and maintain the same concentration relation by multiplying results by two. Such sediments give us always the same concentration factor i.e. they represent a 30 fold concentration of urine.

The next manipulation in order concerns getting a satisfactory sample of sediment on the microscope slide. Here again the questionnaires and literature indicate a rich variety of means by which a routine technical step is performed in different laboratories. For convenience of comment they may grossly be divided into those who attempt to examine all of the sediment and the majority who are content to examine a sample of the sediment. Those who try to examine all of the sediment transfer it to a slide in several different ways. They introduce a pipette through the supernatant urine or decant the supernatant urine and then pour or transfer the sediment with a pipette. When these methods were tried out exactly as described and with different kinds of pipettes managed in different ways it was found impossible to get all of the sediment on the slide by any of them. The texture of the sediment its viscosity and surface tension, to say nothing of numerous extraneous factors like dilution and loss on glass surfaces and wetting qualities prevent this operation being carried out with a reasonable degree of uniformity or accuracy by any of the methods. Other elements of inaccuracy also present themselves in making microscopic preparations of total sediments.

Those who are content to sample sediments employ different kinds of pipettes in various ways for the purpose and many specifically mention capil

lary pipettes What particular advantages capillarity or capillary pipettes afford in sampling urine sediments I have been unable to fathom After seeing some of the pipettes in use and reading the descriptions of the ways they are used, I am unable to come to any other conclusion than that on this point there is very much misunderstanding and confusion

The word capillary comes from the Latin capillus meaning hair, and glass blowers tell me that to them a capillary pipette means one whose walls are very thick in proportion to the bore Such pipettes are less sensitive to temperature changes and therefore better for volumetric measurements From what I can learn, the capillary pipettes alluded to in the questionnaires are not of this type Glass tubes with hairlike bores are sometimes called capillary pipettes because in a restricted way mobile liquids will rise in them by capillary attraction The viscosity of urinary sediments, however, nullifies whatever capillary attraction they may possess for more mobile liquids, and they are positively disadvantageous for handling urinary sediments because the narrowness of their bores, particularly at the tips, tends to break up casts and organized detritus which may be in the sediment They also tend to selectivity in sampling which is even more objectionable Therefore, 2 mm may well be regarded as minimum and 3 mm even better for the bore of pipettes designed for handling urinary sediments

If a pipette or any other kind of a hollow tube, open at the ends, be introduced into a vessel containing a mobile liquid, the liquid rises in the tube to a level even with or perhaps a little higher than the surrounding liquid The liquid in the tube gets to the level of the surrounding liquid by hydrostatic balance, and only that which rises above the level of the surrounding liquid is accounted for by capillarity, a weak and variable force that is hardly ever brought into play in handling urinary sediments The techniques which get up the sediment by introducing a pipette with upper end closed through the supernatant urine until the tip is in contact with the sediment depend upon the rush of urine into the pipette when the pressure of the finger is released to carry along the sediment with it, and not at all upon capillary forces

It is thus evident that capillary pipettes are useless and disadvantageous for sampling urinary sediments Some textbooks and questionnaires give no reasons but specifically warn against suction Nothing has ever occurred in my experience to indicate that suction is any more disadvantageous than other forces brought into play by the different techniques In fact, suction has possibilities of substantial usefulness The real point seems to be that only mild forces should be used in handling urinary sediments and that all forms of violence must be avoided

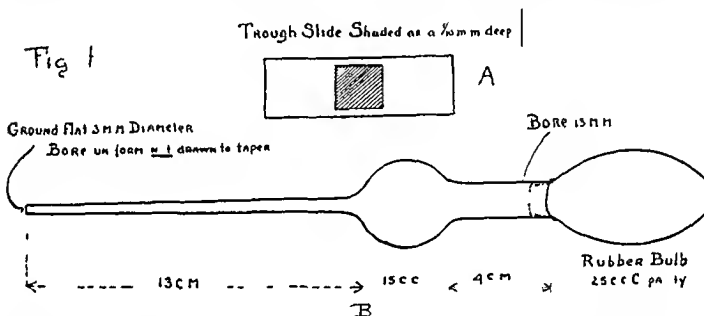
The techniques of the laboratories that regularly examine total sediments are evidently designed to eliminate uncertainties of sampling After practicing the manipulations and taking every precaution to insure uniformity, these techniques were repeated on samples of the same urines and none of them gave reproducible results

The laboratories that sample sediments take different amounts for examination Some stress the importance of mixing sediments before taking sam-

ples, others take samples "from the top, middle and bottom of the sediment with a pipette" and mix them on a slide. When checked experimentally, it was found that mixing the sediment before sampling gives better reproducibility than attempts to sample the sediment by layers and then mix.

Thus urine containing blood cells was centrifuged and samples selected both by picking "representative samples from the top, middle, and bottom of the sediment" and by mixing the whole sediment before selecting the sample. The average deviation between counts of eleven samples selected by the layer technique was 15.5 per cent while the samples selected from the mixed whole sediment deviated only 5.3 per cent.

The only detail concerned with sampling sediments which remains is the method of mixing. Some stir the sediments with a rod, some tap, some rotate and some shake the tube, others blow the sediment in and out of a pipette or jiggle the pipette up and down in the sediment. When checked against one



another no great differences are apparent but the results distinctly favor the last two methods and this finding is corroborated by the whirling currents set up by the other methods which can be clearly seen to cause the microscopic objects to gravitate away from the periphery and form a thin revolving column in the center of the tube.

From the sediment sampling experiments it is clear that the volume of sediment should always be the same and that the sediment or sediment plus urine contained in the suggested 0.5 cc sediment volume constant should be thoroughly mixed before taking the sample for microscopy. To transfer the sample from centrifuge tube to slide the suction pipette (Fig 1) has been found satisfactory and convenient. Its length conforms with the usual 15 cc centrifuge tube. It is made of 3 mm glass tubing plane ground at the tip and dilated near the upper end sufficiently to hold 15 cc. With the rubber bulb compressed the tip is introduced into the centrifuge tube until near the 0.5 cc mark, the supernatant urine is drawn into the pipette by releasing and then ejected by compressing the rubber bulb. The same pipette is then used to mix the sediment and transfer a sample of it to the slide.

MICROSCOPY

Preparing the Specimen

According to the questionnaires 80 of the 100 laboratories employ 3 inch and 20, larger slides in order to accommodate either the entire sediment of 15 c c or multiple specimens. The questionnaires also reveal that 64 laboratories always use cover glasses, that 24 are indifferent about the matter, and that 12 laboratories never use cover glasses for urine sediments. When different techniques outlined in the questionnaires were checked experimentally it was found that the procedure with cover glasses always gave better results than the same procedure without them, and it is, of course, true that even optical and many other considerations decidedly favor the use of cover glasses.

Almost every possible way of preparing a microscopic specimen is included in the methods delineated in the questionnaires, and to try them out in detail required more time and care than all the rest of the work taken together. It is, nevertheless, again necessary to record failure because no matter how painstakingly performed, or how combined, none of the techniques give results which can be fairly called either quantitative, accurate, or reproducible.

In the course of the experiments it became apparent that irregularities and inconsistencies were to some extent at least, due to certain characteristics of sediments which cannot be controlled, such as wetting quality, viscosity, and surface tension. It also became obvious that all microscope counting methods are hopeless unless made under conditions which insure an even volume and thickness of specimen and an even distribution of the microscopic objects therein.

To secure such conditions, Addis, Ockerblad and others resorted to the blood counter, and it was employed extensively throughout this work on urines and sediments for experimental as well as control purposes. In fact it was used so very often that an unusually rich experience in counting was gained from which were derived certain very definite impressions, although sediments were introduced into the counting cell in every conceivable way, from the standpoint of reproducibility our counts continued to be so variable and inconsistent and this experience was taken to indicate that the counter is not as well adapted to sediments as it is to more mobile material like urine and diluted blood. Other features of the blood counter which we think make it less suitable for urinalysis are the rulings which are conspicuous and account for much of its cost, and its very inadequate surface area consequent limitation of content.

For counting the microscopic objects suspended in uncentrifuged urine the blood counter is undoubtedly the best means at our command and is considered standard even though filling and refilling the cell with different samples of the same urine is so tedious and time consuming as to render its employment in routine urinalysis hopelessly impracticable. In this work, for instance, the minimum requirement has been to count 400 squares on a single slide, four different samples of the same well mixed urine even when the objects per c c of the specimen ran into millions and in proportion as the object

specimen grow scarce, it is, of course, well known that more and more counts are necessary not only for accuracy but even to avoid missing their presence altogether

With the idea of trying to dodge its disadvantages and at the same time to utilize the advantages of the blood counter, an experimental cell was constructed which is nothing more or less than a 3 by 1 inch slide having in its middle a 0.1 mm deep trough 400 mm square. Such a trough holds 400 times more sediment than the blood counting cell and is easily and quickly filled, in fact, quicker than by many current methods of preparing specimens because all one has to do is to transfer the sediment to the trough with a pipette and press the 0.5 mm thick cover glass down around the rim of the trough. When made in this way, microscopic preparations always have the same volume and thickness and uniform distribution of microscopic objects throughout the specimen. Table V shows the correspondence of counts made on a series of urines with the blood counter (average of four or more samples) and sediments from the same urines in trough cells (average of 10 fields). The counts were made in the course of the usual routine of the Prudential Laboratory by six different technicians.

TABLE V

COUNTS OF BLOOD AND PUS CELLS IN URINE BY HEMACYTOMETER AND IN SEDIMENTS OF SAME URINE BY NEW METHOD

SPECI MEN	COUNT PER CC URINE			SPECI MEN	COUNT PER CC URINE		
	HEMA CYTOMETER	TROUGH	DIFFERENCE PER CENT		HEMA CYTOMETER	TROUGH	DIFFERENCE PER CENT
pus	82 000	74 000	9.6	blood	62 500	50 900	4.2
blood	50 000	49 000	2.0	blood	52 000	48 000	7.7
pus	95,000	90 000	5.6	pus	37 500	39 200	4.6
pus	160 000	152 000	5.0	blood	12 000	12 000	0.0
blood	27 470	26 700	2.7	pus	37 000	36 100	2.4
pus	27 470	26 700	2.7	pus	42,400	46 200	9.0
pus	40,000	42 000	5.0	pus	14 300	14 000	2.1
blood	25,000	22 400	10.4	blood	7,000	7 000	0.0
pus	280,000	268 000	4.3	pus	145 000	136 400	5.6
blood	270 000	248 000	8.1	pus	50,000	52 600	5.2
blood	17 500	16 500	5.7	blood	115 000	112 000	2.6
pus	15 000	13 300	11.3	pus	15,000	15 400	2.7
blood	20 000	18 000	10.0	pus	20,000	20 200	1.0
blood	45 000	40 300	10.4	blood	25 000	24 100	3.6
blood	76 000	83 300	11.1	pus	17 000	17 100	0.6
pus	22 500	21 000	6.7	blood	12 500	12 200	2.4
pus	17 000	15 400	9.4	pus	10,000	0 800	2.0
pus	125,000	115 500	7.2	pus	17 500	18 200	3.9
pus	142 500	156,700	10.0	pus	450 000	407 000	10.0
blood	87 500	77 400	11.5	blood	255 000	241 500	5.3
Average hemacytometer—trough cell difference 5.6 per cent							

Examining the Specimen

According to the questionnaires, one laboratory tries to examine the entire sediment with both low and high powers, but only rarely are attempts made to cover more fields with high than with low powers. Some cover the same selected fields with both high and low powers but the majority employ high powers only "rarely," "casually," "when needed," "for confirmation," etc. The number of fields regularly examined run all the way from 'several' or five to more than a hundred.

In order to lose no unnecessary time on negative ones, experienced microscopists generally begin their examination of a sediment by carefully but rapidly searching through a number of low power fields. Some such combination as 16 mm objective and 10 power eyepiece giving a magnification of 100 or more suffices for this purpose, and the greater areas covered by low power objectives enable the microscopist to avoid wasting time and tedium over specimens which contain nothing of interest. In his searching the experienced microscopist, if he sees anything which is not clearly and unquestionably distinguishable or recognizable, will resolve it by switching on the high power objective. The technician-microscopist if he has any doubt at all about the identity or significance of an object thus resolved, is duty bound to show it to the director of the laboratory, who, it is assumed, is a pathologist. Technicians should be continually reminded that the principal virtues of a microscopist are keenness and discrimination in spotting unusual and unfamiliar objects and honesty and accuracy in counting objects which have to be counted when they are present in the specimens. Counting objects like casts and pus cells in unstained specimens is tedious work, and the use of higher magnifications than are necessary for comfortably resolving and clearly distinguishing the objects which have to be counted tends to diminish accuracy because of the optical restrictions imposed by higher powers. Similarly operative as another deterrent to accuracy is the thickness of specimen which makes it necessary to focus on objects lying in different planes.

Reporting the Findings

It is now customary to report microscopic findings by numbers counted or by words like rare, many, few, etc. Such words are indefinitely intriguing to many, and it may be possible to conventionalize them so as to convey more or less definite information. Until such conventions are well established, however, very different meanings attach to such words not only among those who report but also by those who interpret them.

On the other hand reports which are made in terms of actual numbers tend to emphasize lack of uniformity and discrepancies, because numbers carry more concrete meanings than words. Also, circumstances such as those outlined in the paragraphs on microscope equipment, sampling, sedimentation, etc., give such reports false air of accuracy which lead to a fool's paradise of misinformation and confusion with consequent irritations and embarrassments.

The remedy for the situation is a change in our habits of reporting. The plan of reporting the number of microscopic objects per unit of urine volume commends itself as being in accord with the nature of urine and having other advantages. It is now customary to report the cellular elements in urine by some equivalent of number per microscope field and to report casts in terms equivalent to the number per slide or total sediment. On the plan suggested all microscopic objects would be reported in the very same terms as so many per cubic millimeter or per c.c. or per 15 c.c. or per liter of urine, as may be preferred. At first sight it appears logical and appropriate to report urine

counts in the same terms as blood counts, but even when clinically significant casts are apt to run less than 1 cast per cubic millimeter, and it would, therefore, be necessary to report them often in terms of a fraction of a cast per cubic millimeter, which is, of course, undesirable. On the other hand, writing more digits as the higher units of volume require is also undesirable. Therefore, the content of 1 cc of urine looks like the best compromise for a satisfactory unit of volume. Table VI has been compiled and calculated to show how simply counts of average sediment fields are convertible into counts per volume of uncentrifuged urine. Thus 20 cells in a high power sediment field equal 62 000 pus cells per cc of uncentrifuged urine and one cast in every tenth low power sediment field equals 17 in 1 cc of the original urine.

TABLE VI

TABLE OF FACTORS FOR CONVERTING MICROSCOPE COUNTS INTO EQUIVALENT NUMBERS PER UNIT OF URINE VOLUME

Field	ACHROMATIC			APOCHROMATIC
	10 OCULAR 4 OBJECTIVE	10 OCULAR 8 OBJECTIVE	10 OCULAR 16 OBJECTIVE	15 OCULAR 8 OBJECTIVE
Diameter	0.37	0.78	1.59	0.55
Surface area	0.108	0.478	1.986	0.238
Volume when depth is 0.1 mm	0.0108	0.0478	0.1986	0.0238
Numbers per unit volume when count is 1 per field				
Per cubic millimeter	93	21	5	42
Per cubic centimeter	93 000	21 000	5 000	42 000
Per 15 cc	1 389 000	313 000	75 000	630 000
Per 15 cc concentrated 30 times (0.5 cc sediment)	40 500	10 500	2 500	21 000
Factor for converting count per sediment field to number in 1 cc urine	3,100	700	170	1 400

Reports of microscopic examinations of urine so often carry the word negative that it deserves to have a specific meaning assigned to it. The answers to the questionnaire suggest 20 low power fields as being generally acceptable. If such a convention were established it would be possible to define a negative specimen as 4 c mm of urine which contained no casts and not enough cellular elements to make it worth while counting them. As a corollary the word negative used in connection with a particular kind of urinary object would mean that there were not enough of them to be of interest in 4 c mm of urine.

Part II

The information developed in Part I enables the formulation of a practicable system of quantitative urinary microscopy. To facilitate its quick and easy performance the centrifuge tubes are scratch marked at levels indicating 0.5 and 15 cc and two pieces of glassware (see Fig 1), a syringe pipette, and a trough slide, have been designed. The pipette conveniently extricates

mixes, and transfers the sediment, the trough slide facilitates counting by insuring a constant volume and thinness of specimen. Fewer fields need be counted than in the usual varied and uneven preparations. If one counts 4 fields, for example, and finds the distribution in the trough as even as it should be, the result multiplied by 5 gives the content of 20 fields*.

To apply the method

- 1 Pour the well mixed urine into the centrifuge tube up to the 15 c c mark, and centrifuge four and one-half minutes at 2000 r p m or the equivalent
- 2 Withdraw the upper 14.5 c c of urine and mix the remaining 0.5 c c of sediment or sediment plus urine with the pipette (juggle up and down)
- 3 With the pipette transfer the sediment to the trough and press the 0.5 mm thick cover glass down around the rim
- 4 Examine the slide under the microscope, and multiply or divide the appropriate factor (Table VI) by the number of objects found in an average field, and report the results as so many blood or other cells or casts in 1 c c of urine

This system of urinary microscopy offers the following advantages

- 1 It is quicker and easier to perform than current methods
- 2 It is sensitive enough for every conceivable purpose
- 3 It enables reporting all microscopic objects in the same terms
- 4 It makes uniformity of reporting by different laboratories possible
- 5 It is much more accurate than current methods
- 6 It is a quantitative method, i e, experimental error around 10 per cent

In conclusion, I desire to express my thanks and indebtedness to Miss Gertrude Orsey and Mr John Huizer for efficient assistance and to Drs P V Wells and Anton R Rose for valuable suggestions and help

Postscript

Since the foregoing paper was written a microscopic slide designed for counting mould cells was brought to my attention by the Bausch & Lomb Optical Company. In its original form it is impractical because unnecessarily cumbersome, complicated and thick. I therefore suggested a simpler and lighter construction which enables the use of high-power objectives and is very practical for urinalysis. The improved mould slide gives exactly the same results as the trough slide but is preferable because easier to make and also because cover glass fit is rendered less messy by a gutter surrounding the specimen which disposes of superfluous

I have taken further advantage of this feature to secure extremely satisfactory slides which hold eight separate sediments under one cover glass. Notwithstanding greater cost such slides are proving actually more economical in routine use than the usual types of microscope slides, and besides other advantages their polished optical glass construction markedly improves the microscopic appearances of colorless objects like hyaline casts.

The technique has been further simplified by automatically measuring the constant sediment volume instead of pipetting off the supernatant urine,

*This statement must not be taken as contradicting the well-known principles that accuracy is greater in proportion to the number of fields counted.

which now drains out through a small hole drilled through the 0.5 cc mark of the centrifuge tube. Before centrifuging cover the hole with a section of rubber tubing and pour an inch or so of water in the cup which holds the centrifuge tube. After centrifuging, remove the rubber (with a small wire hook) and blow gently (breath or bulb) into the tube to overcome slight surface tension effects.

It may also be of interest to note that the examination of some 25,000 specimens by the new method shows very definitely that the correct results obtained greatly increase the clinical value of urinary microscopy.

SUMMARY

Part I

With a view to improving accuracy and uniformity of reporting, current methods of urinary microscopy were checked experimentally and the information developed recorded.

Part II

A simple and rapid quantitative method of microscopic urinalysis is described.

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DISCUSSION

Dr Frederic E. Sondern—Dr Exton's article is most instructive, and the use of quantitative microscopic examinations may give us information we do not obtain from our present methods of estimating the amounts of the different ingredients of the urine sediment. The Rockefeller Institute Hospital of New York has been making its microscopic urine reports in the manner suggested by Dr Exton and I am free to confess that with no experience in this regard they have been difficult to interpret. It will take personal experience to coordinate the new enumeration with the old.

Dr E. R. Mudge—We note that centrifuging always varies with different instruments and the effect of gravity with one instrument will be many times that with another. We have learned with one instrument used in our laboratory that if we allow the centrifuge to run to 2000, there will be a certain distortion of urinary elements. Dr Exton spoke of phosphates, and I presume he meant also amorphous urates. We have been in the habit of removing those urates by warming the specimen, and removing the phosphates by the addition of a small amount of acetic acid in order that we do not get a distortion of the sediment of value and that they can also be seen more readily.

Dr Francis B. Johnson—This point is a very important one particularly with each individual hospital and in hospitals where only interns are employed and to see that some standard be taken by which we can control and compare results. It is interesting that in my own work for a great many years we have used some standards similar to these. We have always centrifuged our urine to a 1:30 concentration. In some cases we have smaller quantities of urine, particularly in children and we make allowances for that. In figuring out how to classify our findings we considered them more in regard to pus. It has been

agreed upon that when there are very few leucocytes found to the high power field under a cover glass we consider that as not positive, so on as 1, 2, 3, and 4. For instance 3 to 10 cells, 1 plus, 10 to 20, 2 plus, 20 to 30, 3 plus, 30 to 40, 4 plus. In looking for casts we felt that using a cover glass made a field so thin that it took too long to find them. The drop was placed on a slide and spread about the same depth as with a cover glass. We use two drops, one with and one without a cover glass.

There has to be a definite method for comparison to be able to check up where a great many men are making these microscopic examinations.

Dr William G. Exton (closing) — May I say that in this work there was so very much repetition of manipulative detail that it was easily the most tedious and troublesome I ever attempted. I am, therefore, gratified to hear what Dr Johnson has said because he has made me feel that the trouble taken was worth while.

As laboratory men we need methods that will enable us to report examination results which agree with one another because we lose the confidence of layman and physician and expose ourselves to criticism when our reports disagree. The method I have outlined for you gives exact and truthful answers instead of the slippery conventionalities we have been hitherto getting. It puts urinary microscopy on a definite quantitative basis and should, therefore, increase the clinical value of urinalyses.

As to some of the suggestions which have been made, I am careful not to add acetic acid or other chemicals to specimens before examining these under the microscope. I prefer to dilute if necessary rather than treat them with reagents which may change the microscopic objects.

The point in centrifuging is to centrifuge long and fast enough to bring the microscopic objects down into the sediment and to stop at this point, because longer centrifuging brings into play packing and other forces which injure the delicate objects we are interested in seeing and counting. I am sorry I omitted reading the part of the paper which deals with injury to microscopic objects, but you will find that centrifugal force is even and steady and not likely to injure the microscopic objects. When they suffer injury it is usually due to packing of the sediment or to forces which are brought into play in extricating the sediment from the supernatant urine. Dr Sondern is very much to the point about the matter of coordinating correct enumeration with our present system.

ACUTE DIFFUSE MYELITIS FOLLOWING INTRAVENOUS INJECTION OF NEOARSPHENAMINE*

BY ERNEST SCOTT, M D, AND HARRY L. REINHART, M D, COLUMBUS, OHIO

THE report of the salvarsan committee of the British Medical Research Council on the toxic effects following the employment of arsenobenzol preparations is the most exhaustive compilation of facts and observations upon this subject in the literature today, and it is indeed pertinent that their conclusions are headed by the statements that 'no special arsenobenzol preparation can be regarded as more likely than others to produce ill effects. Errors in technic cannot account for more than a few serious accidents, fatalities have occurred even under the most careful control in large and completely equipped hospitals''

From considerable experience and observation with various arsenobenzol preparations, and their administration under both the best and the worst technic, we are of the opinion that there is a wide range of tolerance in most individuals, and that the nature of the preparation and the technic are of less importance in the fatalities which may follow the administration of arsenobenzol preparations than the tolerance of the patient. It is for this reason that it is always advisable to begin a course of salvarsan administration with minimal dosage and increase it gradually to the therapeutic dose, observing the patient most carefully meanwhile. Even then we are not assured that we will encounter no fatalities. On the other hand, physicians should not so readily assume that their technic or favorite preparation is omnipotent in the prevention of fatalities, since the incidence of fatalities is about one in each 5,000 to 10,000 injections, or according to the German statistics, as cited by Phelps,¹ one death for every 3,788 cases treated. From such statistics it is at once apparent that most physicians may administer salvarsan preparations throughout their lives without encountering a single fatality.

Continuing the summary of the British Medical Research Council² the following are listed as the most important ill effects which may end fatally

- (a) "Encephalitis hemorrhagica,
- (b) 'Acute yellow atrophy of the liver,
- (c) 'Exfoliative dermatitis and its complications'

It is further pointed out that encephalitis hemorrhagica is most frequently described in the German literature and exfoliative dermatitis and its septic complications have accounted for most of the fatal accidents in the British and American literature.

To the above list of ill effects which may follow the administration of salvarsan, and which not infrequently result in death we would add another con

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agreed upon that when there are very few leucocytes found to the high power field under a cover glass we consider that as not positive, so on as 1, 2, 3, and 4. For instance 3 to 10 cells, 1 plus, 10 to 20, 2 plus, 20 to 30, 3 plus, 30 to 40, 4 plus. In looking for casts we felt that using a cover glass made a field so thin that it took too long to find them. The drop was placed on a slide and spread about the same depth as with a cover glass. We use two drops, one with and one without a cover glass.

There has to be a definite method for comparison to be able to check up where a great many men are making these microscopic examinations.

Dr William G. Exton (closing).—May I say that in this work there was so very much repetition of manipulative detail that it was easily the most tedious and troublesome I ever attempted. I am, therefore, gratified to hear what Dr Johnson has said because he has made me feel that the trouble taken was worth while.

As laboratory men we need methods that will enable us to report examination results which agree with one another because we lose the confidence of layman and physician and expose ourselves to criticism when our reports disagree. The method I have outlined for you gives exact and truthful answers instead of the slippery conventionalities we have been hitherto getting. It puts urinary microscopy on a definite quantitative basis and should, therefore, increase the clinical value of urinalyses.

As to some of the suggestions which have been made, I am careful not to add acetic acid or other chemicals to specimens before examining these under the microscope. I prefer to dilute if necessary rather than treat them with reagents which may change the microscopic objects.

The point in centrifuging is to centrifuge long and fast enough to bring the microscopic objects down into the sediment and to stop at this point, because longer centrifuging brings into play packing and other forces which injure the delicate objects we are interested in seeing and counting. I am sorry I omitted reading the part of the paper which deals with injury to microscopic objects, but you will find that centrifugal force is even and steady and not likely to injure the microscopic objects. When they suffer injury it is usually due to packing of the sediment or to forces which are brought into play in extricating the sediment from the supernatant urine. Dr Sondern is very much to the point about the matter of coordinating correct enumeration with our present system.

ACUTE DIFFUSE MYELITIS FOLLOWING INTRAVENOUS INJECTION OF NEOARSPHENAMINE*

BY ERNEST SCOTT, M D, AND HARRY L. REINHART M D, COLUMBUS, OHIO

THE report of the salvarsan committee of the British Medical Research Council on the toxic effects following the employment of arsenobenzol preparations is the most exhaustive compilation of facts and observations upon this subject in the literature today, and it is indeed pertinent that their conclusions are headed by the statements that "no special arsenobenzol preparation can be regarded as more likely than others to produce ill effects. Errors in technic cannot account for more than a few serious accidents, fatalities have occurred even under the most careful control in large and completely equipped hospitals."

From considerable experience and observation with various arsenobenzol preparations, and their administration under both the best and the worst technic, we are of the opinion that there is a wide range of tolerance in most individuals and that the nature of the preparation and the technic are of less importance in the fatalities which may follow the administration of arsenobenzol preparations than the tolerance of the patient. It is for this reason that it is always advisable to begin a course of salvarsan administration with minimal dosage and increase it gradually to the therapeutic dose, observing the patient most carefully meanwhile. Even then we are not assured that we will encounter no fatalities. On the other hand, physicians should not so readily assume that their technic or favorite preparation is omnipotent in the prevention of fatalities, since the incidence of fatalities is about one in each 5,000 to 10,000 injections, or according to the German statistics, as cited by Phelps¹ one death for every 3,788 cases treated. From such statistics it is at once apparent that most physicians may administer salvarsan preparations throughout their lives without encountering a single fatality.

Continuing the summary of the British Medical Research Council² the following are listed as the most important ill effects which may end fatally

- (a) 'Encephalitis hemorrhagica,
- (b) "Acute yellow atrophy of the liver
- (c) 'Exfoliative dermatitis and its complications'

It is further pointed out that encephalitis hemorrhagica is most frequently described in the German literature and exfoliative dermatitis and its septic complications have accounted for most of the fatal accidents in the British and American literature.

To the above list of ill effects, which may follow the administration of salvarsan, and which not infrequently result in death, we would add another con-

*Read before the Eighth Annual Convention of the American Society of Clinical Pathologists, Portland, Oregon, July 5, 8, and 9, 1929.
Department of Pathology, Ohio State University, Columbus, Ohio.

dition which while rare nevertheless is definite in its clinical and pathologic aspects. This condition is characterized by a *diffuse acute degeneration of both the white and grey substance of the spinal cord, with marked destruction of the ganglion cells of the anterior horns*. Clinically, we have designated it acute myelitis, pathologically it resembles the hemorrhagic encephalitis which follows salvarsan injection, and like the latter condition it has been reported more frequently in the German literature than in the British or American literature. In fact, after careful search through the British and American literature of the last ten years we have been unable to find a single case which clinically has followed the course of this condition, and which has been carefully checked by autopsy.

The following case illustrates the clinical course of a case of acute myelitis following the intravenous administration of neosalvarsan.

CASE REPORT

E. A., twenty-three years old white, male, married, well developed and nourished, entered the office of a private physician and requested a blood test. He did not complain of any particular symptoms, nor did he give his reasons for desiring a blood test. There was neither a history of luetic infection, manifested by a chancre, generalized rash or sore throat, nor on physical examination was there any evidence of active or latent syphilis. The report of the blood Wassermann was strongly positive for syphilis. He was immediately given an intravenous injection of 0.9 gm. of neoarsphenamine, during and after which he manifested no signs or symptoms of any sensitivity to the drug. Five days later he was given a second intravenous injection of 0.9 gm. of neoarsphenamine, during the administration of which he manifested no unusual symptoms. Approximately eight hours later he complained of a cold and was administered some cold medicine. Two days later he felt some better, except for general weakness and numbness of the feet and ankles. The following day the weakness and numbness of the lower extremities had extended to the hips. Upon examination he showed a loss of sensation up to the hips and marked weakness of the lower extremities, which amounted to a paralysis. In consultation with two other physicians, opinions differed as to the nature of the process, one being inclined to believe that it was an acute syphilitic, transverse myelitis, while the other believed it to be an acute anterior poliomyelitis. Thus, there was clinical evidence of a process involving both the white and grey substance of the cord. The process advanced rapidly until eleven days after the last injection of neoarsphenamine when he entered a hospital the following notations were made. There was a complete flaccid paralysis of the body and lower extremities, while the upper extremities exhibited a paresis and paresthesia of the hands. The biceps reflexes were barely perceptible, all others being abolished. There was evidently a paralysis of the intercostal muscles as the breathing was abdominal in type, attended by a diminution in size of the chest on inspiration and expansion on expiration. Loss of sensation extended up to the second rib anteriorly and second dorsal spine posteriorly. The bladder was distended and at the level of the umbilicus. The sensorium was fairly clear at times.

The hospital laboratory reports were as follows. The blood Wassermann was negative, the spinal fluid Wassermann was positive, 2 plus, and the cell count of the spinal fluid was 19. The blood count revealed 4,200,000 erythrocytes with a hemoglobin of 78 per cent, the total leucocyte count was 14,880 with a differential count of 80 per cent polymorphonuclears, 13 per cent lymphocytes and 7 per cent mononuclears. The urine showed a 2 plus albumin with many polymorphonuclear leucocytes and erythrocytes.

A summary of the clinical course revealed a young man without definite clinical evidence of syphilis who following the second injection of neoarsphenamine, developed a reaction apparently due to the neoarsphenamine, which indicated injury to the spinal cord as manifested by an extensive flaccid

paralysis and complete loss of sensation below the cervical cord, and rapidly progressed to a fatal termination within two weeks. The clinical diagnosis was arsenical, or luetic endarteritis of the vessels of the cord.

The autopsy was held the day following death, the body having been embalmed in the meantime. The anatomic diagnosis at the autopsy was (1) edema of lungs and slight hydrothorax, (2) nephrosis, (3) splenomegaly, (4) dilatation of urinary bladder, (5) bilateral dilatation of the pelvis of the kidneys, (6) left suppurative pyelitis, (7) edema of brain, (8) acute diffuse myelitis, and (9) multiple decubital ulcers of back and sacrum. Primary cause of death was acute diffuse, toxic myelitis following intravenous injection of neosalvarsan, secondary cause was general sepsis.



Fig 1.—Marchi stain. Diffuse degeneration of myelin sheaths of all tracts and multiple hemorrhages scattered throughout the cord. Both white and grey substance is involved and there is no inflammatory reaction present.

Since the clinical symptoms indicated definite injury to the spinal cord it was examined with much interest. The vertebrae and the spinal canal were normal. The dura presented no pathologic lesions, nor was there any gross evidence of hemorrhage or thrombosis of the vessels of the cord. The consistency of the cord was decidedly pathologic, being so soft that it was impossible to section it cleanly with a sharp knife without crushing it. This condition was present from the lumbar region to the upper cervical region where it gradually approached a normal consistency. The surface of the cut section was moderately hemorrhagic but without evidence of suppuration or formation.

Microscopic examination of the cord revealed a moderate edema and vascular congestion of the pia. There was no evidence of exudation or local proliferation of cells in the pia. The dorsal roots presented a slight, sparse degeneration of the myelin sheaths. The white substance of the cord was

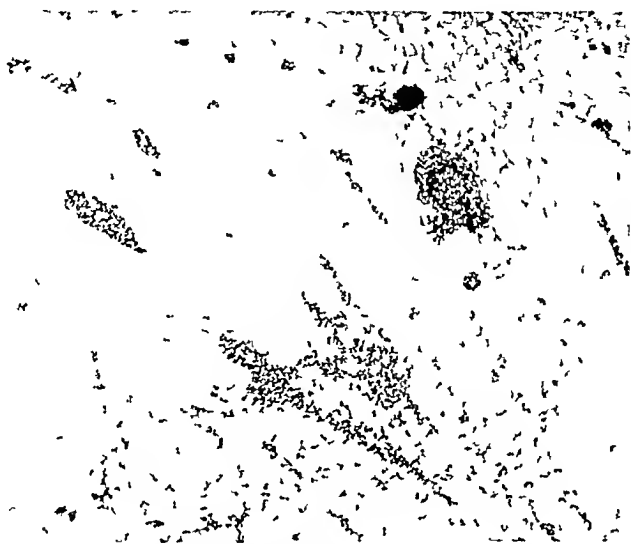


Fig 2—Columns of the cord showing vascular congestion hemorrhage and hyaline degeneration of the walls of the blood vessels. There is considerable perivascular hemorrhage but no perivascular infiltration of leucocytes.

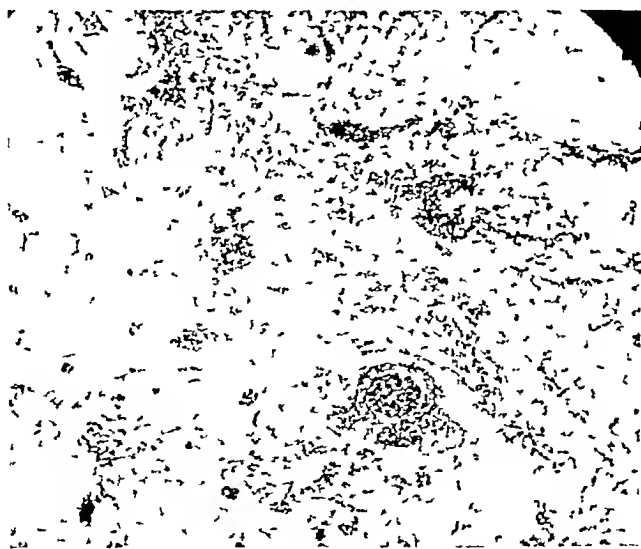


Fig 3—Anterior horn showing complete degeneration of anterior horn ganglion cells, vascular congestion and hemorrhage.

markedly and diffusely degenerated. Practically every tract was extensively involved by this degeneration, which was all the more striking by the absence of any inflammatory reaction (Fig 1). This degeneration extended from the lumbar to the cervical region, being most marked in the upper dorsal and

lower cervical region. The blood vessels were in various stages of degeneration, as manifested by edema of the cells of the walls, hyaline degeneration, thrombosis, congestion and rupture with perivascular hemorrhages (Fig 2). The grey substance of the cord was likewise involved in the degeneration, the most striking pathology being the complete destruction of the ganglion cells of the anterior horns.

The picture in general conformed to the type of microscopic pathology seen in acute hemorrhagic encephalitis following the administration of salvarsan. Comparison of the cord pathology of this case with the brain pathology of the case of acute hemorrhagic encephalitis reported by Scott and Moore³ revealed a striking similarity in type and distribution of the lesions. In many of the necropsies where a hemorrhagic encephalitis has been found following the administration of arsphenamine the cord has not been examined which is unfortunate, since it does not reveal whether or not these two lesions are ever associated. In the present case there was no similar associated pathology in the brain.

A summary of the pathology of the cord reveals a diffuse myelomalacia involving both white and grey substance, degenerative lesions of the blood vessels with perivascular hemorrhages, and complete destruction of all ganglion cells of the anterior horns (Fig 3).

DISCUSSION

It is at once apparent that the most probable etiologic agents in this case are either syphilis or neoarsphenamine. The only evidence of syphilis is a positive blood Wassermann reaction in one laboratory test—a negative blood Wassermann in another following neoarsphenamine administration, and a positive spinal fluid Wassermann after the acute pathology had developed. There was no history or clinical evidence of syphilis. The gross and microscopic examinations of the various organs removed at autopsy presented no lesions which were even suggestive of syphilis. Therefore, we have at least eliminated syphilis pathologically and feel that the evidence for syphilis is minimal.

In classing this case as another one of those rare fatalities which occasionally follow the injection of arsenohenzol preparations, we note many points of similarity in the clinical course and pathology. Thus, for example, the reactions after intravenous arsenic preparations may be classed as immediate or delayed. In the case under discussion the reaction was not immediate but delayed. It occurred within twelve hours after the second injection of neoarsphenamine which is the time of greatest incidence of delayed reactions. Furthermore, the delayed reactions are essentially cases of arsenic poisoning, and the pathology in this case is essentially that of arsenic poisoning.

Chiari⁴ cites two cases of myelitis following poisoning with arsenous acid. He says that examination of the cords revealed degeneration of the ganglion cells in the anterior horns with atrophy of the medullated network in the anterior horns and poverty of the fibers in the anterior columns, * * * * degeneration of Goll's columns in the cervical marrow and a circumscribed hemorrhage in the left anterior horn in the second lumbar segment. Chiari continues with a report of the cord pathology following the intravenous

administration of neosalvarsan as follows, severe disease of the seventh to ninth thoracic segments of the cord from which both ascending and descending degeneration passed out. This disease was not a true myelitis, such, for example, as that of epidemic poliomyelitis. It was rather a regressive change of the character of a necrosis which had affected both grey and white substance. There was marked destruction of ganglion cells, and the medullated network in the grey substance was entirely degenerated. Chiari is, therefore, of the opinion that his case of myelitis following the intravenous administration of neoarsphenamine was caused by the arsenic component of the drug. The similarity of the pathology of the two cases of arsenic poisoning, as well as the myelitis following salvarsan, agrees entirely with the pathology of our case.

Nonne⁵ reports a fatal injury to the spinal cord after intraspinal salvarsan treatment, in which the predominant lesion was degeneration. He cites this case as the fourth case of severe incurable disease of the spinal cord after intraspinal use of salvarsan, and, while the other three cases were not autopsied, the symptoms were those of paralysis of the *conus medullaris*.

Mingazzini⁶ also reports a hemorrhagic postsalvarsan myelitis, in the discussion of which he cites Nonne's case and states that it and his own case are two really classical cases of postsalvarsan myelitis in which the clinical symptomatology and the postmortem findings were in harmony. Our own case is in entire accordance with all cases cited, and conforms to the classical clinical course, and cord pathology.

ETIOLOGY

Many theories have been presented to explain the action of arsphenamine in the fatalities which have followed its administration. An excellent summary of these is given in the paper by Scott and Moore.³ We should like to repeat this summary of the pathologic lesions as found in cases of "(1) experimental poisoning of animals, (2) the lesions found at autopsy in individuals who have died from poisoning with inorganic arsenic preparations, (3) those in which fatalities followed the administration of salvarsan."

"1. A destructive action on the endothelium resulting in congestion and hemorrhage in most of the organs of the body.

"2. The poor excretion resulting in edema of the brain and lungs in particular.

"3. A nephritis, usually of the tubular type, but under certain circumstances taking on also a glomerular type.

"4. The findings of infarcted and necrotic areas in the various viscera in some cases.

"5. Necrosis of the liver, and sometimes acute yellow atrophy."

Besides the characteristic pathologic picture of arsenic poisoning in the spinal cord our case presented evidence of arsenic poisoning in the destruction of the vascular endothelium and congestion, edema of the brain and lungs, and a nephrosis.

We are therefore, of the opinion that the essential pathology is the result of the arsenic component of the neoarsphenamine. While it may be ques-

tioned that the results in this case would have been otherwise still we believe that all administrations of salvarsan should begin with minimal dosage and slowly progress to the therapeutic dosage

SUMMARY

1 A classical case of acute myelitis following the intravenous administration of neosalvarsan, in which both clinical and pathologic findings coincide, is presented

2 The clinical course is that of an acute onset of extensive flaccid paralysis rapidly following the second intravenous administration of neoarsphenamine and terminating in death in less than two weeks

3 The pathologic cord findings are those of extensive degeneration of blood vessels diffuse degeneration of white and grey substance of the cord and complete destruction of ganglion cells of the anterior horn, without exudate phenomena or glial proliferation

4 Such cases should be classed along with hemorrhagic encephalitis as one of the potential causes of fatalities following the intravenous administration of arsphenamine

REFERENCES

- 1 Phelps J R A Fatal Case of Acute Poisoning by Neoarsphenamine Comment U S Nat M Bull 22 217 1925
- 2 Report of the Salvarsan Committee II Toxic Effects Following the Employment of Arsenobenzol I reparations Medical Research Council Special Report Series No 66 1922
- 3 Scott and Moore Fatalities Following the Use of Arsphenamine J LAB & CLIN MED 13 345 1928
- 4 Chauri H Über eine nach Neosalvarsaninjektionen aufgetretene Myelitis Verhandl d deutsch path Gesellsch 155 1913
- 5 Nonno M Letale Rückenmarks schädigung durch intraspinal Salvarsanbehandlung Deutsche Ztschr f Nerven 94 158 1926
- 6 Magazzini G Klinischer und pathologisch anatomischer Beitrag zum Studium der Myelitis haemorrhagica postsalvarsanica Deutsche Ztschr f Nerven 104 1 1929

TRANSACTIONS

Minutes Eighth Annual Convention American Society of Clinical Pathologists Portland, Oregon, July 5, 6, and 8, 1929

The proceedings were held in the Portland Hotel, Portland, Oregon, July 5, 6, and 8, 1929

The meeting was called to order Friday, July 5, 1929, at 9 A.M. by the President Elect, Dr J H Black, Dallas, Texas Dr H H Foskett, Portland, Oregon, Chairman of the Local Committee on Arrangements, made several announcements regarding entertainment planned for the Members The proposed amendments to the Constitution and By Laws were introduced and a motion was made and carried that they be brought up at the proper time Monday

There being no further business at this time the following program was presented at the regular scientific session of the Society

"The Effect of the Presence of Bile on the Agglutination Reaction" By Ruth Gilbert, M.D., and Marion B Coleman, B.S., Albany, New York Read by Dr V W Bergstrom No discussion

"Recent Developments in Tularemia (Francis' Disease) With a Report of Eleven Additional Cases" By Walter M Simpson, M.D., Dayton, Ohio Discussion by Dr J C Geiger, San Francisco, Dr W T Cummins, San Francisco, Dr C W Bonyng, Los Angeles, Dr Warren T Vaughan, Richmond, Virginia, Dr Robert F E Stier, Spokane, Washington, Dr Roy W Hammack, Los Angeles, Dr Frederic E Sondern, New York City, and Dr Walter M Simpson, Dayton, Ohio

"The Routine Use of Photoelectric Hemoglobinometer" By A H Sanford, M.D., and Charles Sheard, Ph.D., Rochester, Minnesota Discussion by Dr Walter M Simpson, Dayton, Ohio, and Dr A H Sanford, Rochester, Minnesota

"Acute Diffuse Myelitis Following Intravenous Injection of Neoarsphenamine" By Ernest Scott, M.D., and H L Reinhart, M.D., Columbus, Ohio Read by Dr C H Fanlove, Portland, Oregon No discussion

"Spontaneous Meningeal Hemorrhage" By Frederick H Lamb, M.D., Davenport, Iowa Discussion by Dr A S Giordano, South Bend, Indiana, and Dr Frederick H Lamb

Friday Afternoon, July 5, 1929, 2 p m

The meeting was called to order by the President, Dr F W Hartman who appointed a Nominating Committee as follows Dr Rawson J Pickard, Chairman, San Diego, California, Dr Robert A Keilty, Washington, D C, and Dr Walter M Simpson, Dayton, Ohio To fill vacancies on the Research Committee President Hartman appointed the following Fellows Dr Frederic E Sondern, New York City, Dr W T Cummins, San Francisco, and Dr C W Maynard, Pueblo, Colorado The scientific program was continued

"Undulant Fever in Man A Clinical Analysis of Thirty Three Cases" By A S Giordano, M.D., and R L Sensesch, M.D., South Bend, Indiana

"The Etiology and Diagnosis of Undulant Fever in the United States" By Charles M Carpenter, M.D., and Ruth Boak, Ph.D., Ithaca, N Y (By invitation)

"Some Observations on the Agglutination of B Abortus" By Frank B Lynch, Jr, M.D., and Annette M Callan Philadelphia, Pa Read by Dr Ralph Mills, Rochester, Minn

"Notes on the Bacteriology of the Brucella Group" By K F Meyer, M.D., and B Eddie, San Francisco, California Read by J C Geiger, M.D., San Francisco, California (By invitation)

Papers discussed by Dr Walter M Simpson, Dayton Ohio Dr F W Hartman Detroit Dr J C Geiger, San Francisco Dr C W Mynard Pueblo Colorado Dr R L Sensenich South Bend Indiana Dr A V Hardy Iowa City Iowa Dr Roy W Hammack Los Angeles Dr C W Bonyng Los Angeles Dr D Schuyler Pulford Woodland California Dr E R Mugrage Denver Dr A S Giordano South Bend, Indiana and Dr Charles M Carpenter, Ithaca, N Y

Friday Evening, July 5, 1929, 7 pm Round Table Discussion

The meeting was called to order by President F W Hartman The first subject for discussion was presented by Dr Philip Hilkowitz Denver entitled 'Virtuosity in Clinical Pathology' Discussion by Dr F W Hartman Detroit, Dr William G Exton Newark N J Dr Frederiek H Lamb Davenport Iowa, Dr Frederic E Sondern New York City and Dr Philip Hilkowitz

Problems By W G Gamble Jr, M D Chicago Illinois Discussion by Dr Wilham G Exton Newark Dr Frederic E Sondern New York Dr Charles R Drake Minneapolis, Dr Warren T Vaughan, Richmond, Va Dr B W Rhamy Fort Wayne Indiana, Dr Zera E Bolin, San Francisco Dr Philip Hilkowitz Denver Dr Francis B Johnson Charleston S C Dr Roy W Hammack Los Angeles Dr C W Bonyng Los Angeles and Dr W G Gamble Chicago

'The State Laboratory Problem' By Frederic E Sondern New York City Discussion by Dr B W Rhamy Fort Wayne and Dr Frederic E Sondern

"The Hospital Situation"

A Scientific By Robert A Keilty M.D, Washington D C

B Statistical By Philip B Matz M.D, Washington D C read by Dr A H Sanford Rochester, Minnesota

C Relation to the American College of Surgeons By J J Moore, M D Chicago Read by Dr W G Gamble Jr Chicago

Discussion by Dr A H Sanford Rochester Dr Frederic E Sondern New York City Dr Robert A Keilty Washington D C and Dr F W Hartman Detroit

'Is the Cost of Laboratory Work Too High? By Robert F E Stier M.D Spokane Washington. Discussion by Dr Mortimer Herzberg Cincinnati Ohio

The Clinical Pathologist in the Rural Hospital' By C W Maynard M.D Pueblo Colorado Discussion by Dr W T Cummins San Francisco Dr Zera E Bolin San Francisco and Dr Francis B Johnson Charleston S C

Saturday Morning, July 6, 1929, 9 am

The meeting was called to order by President Hartman and the scientific program continued

Gingivitis V The Character of the Exudate in Gingivitis By Robert A Keilty M.D, Washington, D C Discussion by Dr M M Patton Seattle Washington Dr F H Lamb Davenport Iowa Dr E C Rosenow Rochester Minnesota Dr F W Hartman Detroit and Dr Robert A Keilty

'The Tuberculous Cavity By Alfred Blumberg M.D Oteen N C Read by Dr E R Mugrage Denver Colorado Discussion by Dr F W Hartman Detroit Michigan and Dr Walter M Simpson Dayton Ohio

Oxalic Acid as a Reagent for Isolating Tubercle Bacilli and a Study of the Growth of Acid fast Nonpathogens on Different Mediums With Their Reactions to Chemical Reagents By H J Corper M.D and Nuo Uyer Ph.D Denver Colorado Discussion by Dr Philip Hilkowitz, Denver Dr Frederic E Sondern New York Dr A S Giordano South Bend, Indiana Dr E C Rosenow Rochester Minnesota Dr Robert A Keilty Washington D C and Dr H J Corper

'A Recently Isolated Bacillus of the Hemophilic Group By Frank W Hartman M.D and Edna Jackson M.S Detroit Michigan No discussion

"Immunological Specificity of Green Producing Streptococci Having Elective Localizing Power as Isolated in Various Diseases" By E C Rosenow, M D, Rochester, Minnesota Discussion by Dr Robert A Kelty, Washington, D C, and Dr E C Rosenow

Saturday Afternoon, July 6, 1929, 2 p m

The meeting was called to order by President Hartman and the scientific program continued

"Milk Borne Rabies" By E R Mugrage, M D, Denver, Colorado No discussion

"Observations on Intestinal Protozoiasis" By Rawson J Pickard, M D, San Diego, California Discussion by Dr Z E Bohm, San Francisco, and Dr Rawson J Pickard

"Pathology of the Reticulo Endothelial System" By Zera E Bohm, M D, San Francisco, California Discussion by Dr E R Mugrage, Denver, and Dr Z E Bohm

"Reticulocytes, Their Identification and Significance" By C L Spohr, M D, Columbus, Ohio Read by title

"Improved Colorimetric Procedures for the Quantitative Estimation of the Proteins of the Cerebrospinal Fluid" By Philip B Matz, M D, Washington, D C, and Nathan Novick, Hines, Illinois Read by title

"Quantitative Microscopic Urinalysis" By William G Exton, M D, Newark, N J Discussion by Dr Frederic E Sondern, New York, Dr E R Mugrage, Denver, Dr Francis B Johnson, Charleston, S C, and Dr William G Exton

"New Quantitative Clinical Methods for the Junior Scopometer" 1 Protein in Urine, 2 Protein in Blood, 3 Protein in Spinal Fluid, 4 Globulin in Urine, 5 Sugar in Urine, 6 Sugar in Blood, 7 Urea in Urine, 8 Ammonia in Urine, 9 Creatinin in Urine, 10 Sulphur Partition in Urine By William G Exton, M D, Anton R Rose, Ph D, and P V Wells, D Sc, Newark, N J (Read by title)

"Embryonal Carcinoma of the Testicle" By L W Larson, M D, Bismarck, N D

"Malignant Tumors of the Testicle" By O A Brines, M D, Detroit, Michigan Discussion of both papers by Dr Charles R Drake, Minneapolis, Dr Zera E Bohm, San Francisco, Dr Roy W Hammack, Los Angeles, Dr L W Larson and Dr O A Brines

Saturday Evening, July 6, 1929, 7 p m Annual Banquet

The Annual Banquet was held in the ballroom of the Portland Hotel The speakers of the evening were as follows

"Presidential Address" By Dr Frank W Hartman, Detroit, Michigan

"Address" By Dr R G Coffey, Portland, Oregon

"Address" By Dr Cyrus C Sturgis, Ann Arbor, Michigan

"Presentation of the Ward Burdick Research Award" The award was made by President Frank W Hartman to Dr Walter Malcolm Simpson, Dayton, Ohio, for his work in Tularemia

Monday, July 8, 1929, 9 a m Business Session

The meeting was called to order by President Frank W Hartman The reading of the minutes of the previous meeting were dispensed with since they had previously been published

The report of the Executive Committee was made by the Chairman, Dr A H Sanford, Rochester, Minnesota Dr Sanford reported progress and that his Committee had selected Portland as the 1929 meeting place, they had inspected the audit of the books of the Treasurer and found it correct Report accepted

The report of the Editorial Committee was made by Dr Robert A Kelty, Washington, D C, Associate Editor, by reading a communication from the Editor in Chief, Dr T B Magath, Rochester, Minnesota, stating that our relations with the publishers of THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE have been satisfactory, that they would like to receive editorials for the Journal from members of the Society, that the Round Table Discussions be abstracted for publication and that authors scrutinize their bibliographic references more carefully and abbreviate their manuscripts as much as possible Report accepted

The Report of the Publication Committee by the Chairman, Dr John A Kolmer, Philadelphia, was read by Dr H J Corper, Denver, Colorado This Committee reported progress made during the past year in the preparation of the book on Standard Methods in Clinical Pathology Different sections will be submitted to different committees of the Society for revision so that the finished book will represent in truth and in fact methods approved by the Society The authors hope the book will be authoritative and make the American Society of Clinical Pathologists' endorsement stand for the very highest in clinical pathology Nothing further has been done regarding the publication of a journal by the Society but a letter from Williams and Wilkins Company shows their continued interest in this enterprise and offers their assistance in the project Report accepted

Dr Philip Hilkowitz, Denver, Chairman of the Board of Registry read the report of the Committee on the Registration of Technicians stating that steps had been taken to organize and formulate the plans for enrrying the aims and purposes of the Registry into execution in accordance with instructions given at the last annual convention in Minneapolis Application blanks had been drawn up a booklet explaining the scope of the Registry was formulated and distributed widely publicity was given the formation of the Registry by write ups in the medical press of the country There has been received up to this time over five hundred applications for registration which are being carefully examined and certificates will be issued to those successfully meeting the requirements of the Registry The Committee is gratified at the favorable reception that has been accorded its efforts and the numerous letters of commendation and praise that have come both from applicants as well as clinical pathologists Registration of approved training schools for technicians is also under consideration and a survey will shortly be made The Employment Service in this connection has been functioning with success A gold pin has been designed and which is available to those technicians meeting the requirements Thanks were expressed to the members of the Society for their kind interest and cooperation to the state representatives of the Registry and to the Board of Registry for the generous aid they have extended to the Chairman in the conduct of the Registry This department has been a pronounced success both from the standpoint of the clinical pathologist as well as of the laboratory technician Report accepted

The report of the Committee on Public Relations by Dr Edward F Cooke, Chairman Houston Texas, was read by Dr R E Myers Oklahoma City Oklahoma and stated that many and serious evils are attacking the branch of medicine clinical pathology that it fails to attract young men, that many men in the field are leaving it for more lucrative fields As a remedy for the Public Health Laboratory situation it was suggested that these laboratories should be restricted to strictly epidemiologic work and that this work should come only through Public Health Officers and should not be available to any and every practitioner of medicine Clinical pathologists have been approached by the American Medical Association to carry ads in the Medical Directory yet no other branch of medicine would even be allowed to run such ads The Committee also asks why the American Medical Association should approve laboratories when they take no steps like this in regard to any other specialty of medicine An examination of the Medical Practice Acts of the forty eight states reveals that in twenty nine the statutes do not fully define the practice of medicine making it possible for an illegal practitioner to open a laboratory charge for the laboratory examination, and give medical advice free The use of the technician by physicians in their private laboratories is one of the most potent elements in the continuance of the evil all this making clinical pathology an undesirable field for physicians Resolutions drawn up by the Committee were read and referred to New Business Report accepted

The report of the Service Bureau Committee was made by Dr H J Corper Chairman, Denver Colorado Dr Corper reported progress and expressed the conviction that in time this department of the American Society of Clinical Pathologists will be a very useful feature in placing our Fellows in the most desirable locations in the country and acting in a capacity of consultant and informant to the various institutions bringing their problems to us for solution Report accepted

The report of the Research Committee was made by Dr A S Giordano, South Bend, Indiana, for the Chairman, Dr Alvin G Foord, Buffalo, N Y, stating that this Committee endeavored to stimulate joint study of "Undulant Fever" and a symposium was planned for this meeting. In regard to the presentation of the Ward Burdick Award it was felt by the Committee that possibly it would be better to judge the papers as they are presented at the Convention but that the Executive Committee take the matter under consideration. The suggestion was made that the study of "Undulant Fever" be continued for another year in addition to some other subject. Report accepted.

The report of the Committee on Exhibits was made by Dr C H Manlove, Chairman, Portland, Oregon. Dr Manlove stated that their Committee had obtained eight exhibits in all and recommended that the next Chairman start his plans earlier. Report accepted.

The report of the Committee on Necrology was presented by Dr J H Black, Chairman, Dallas, Texas, who reported the death of four Fellows of the American Society of Clinical Pathologists during the past year as follows: Dr Joseph R Losee, New York City, Dr Carl O E Werner, San Francisco, Dr John Hewat, New York City, and Dr M L Holm, Lansing, Michigan. A motion was made and carried that the Society rise a moment in respect to the members that we have lost, and accordingly the members present arose. Report accepted.

The report of the Board of Censors was made by Dr C H Manlove, Chairman, Portland, Oregon. The following were elected to Fellowship in the Society: Dr Arthur L Amolsch, Detroit, Dr Walter G Bain, Springfield, Illinois, Dr O A Brines, Detroit, Dr Henry T Brooks, New York City, Dr Theodore R Helmbold, Pittsburgh, Dr R C Henderson, Perry Point, Maryland, Dr Frank P Hunter, Lafayette, Indiana, Dr Hugh Jeter, Oklahoma City, Dr Frank L Kelli, Philadelphia, Dr Harry K Langdon, Indianapolis, Ind. One reinstatement: Dr W W Hall, Watertown, N Y. One Associate Member, Dr Willa M F Davis, Washington, D C.

Under New Business was brought up the resolutions presented by the Committee on Public Relations. The first resolution stating that the Medical Practice Acts of twenty-nine states fail to properly define the Practice of Medicine as it exists today and in no state in the Union is there provision for any penalty for employing or covering illegal Practitioners of Medicine and resolves that the American Society of Clinical Pathologists in regular meeting assembled deplores this condition, calls the attention of the American Medical Association to it and urges relief. Resolution accepted. The resolution regarding Public Health Laboratories was defeated. A third resolution, that since there is an unfortunate tendency to regard clinical pathology as being independent and apart from the practice of medicine and to provide rules of ethics and conduct apart from those that are supposed to govern practitioners of medicine it is resolved by the American Society of Clinical Pathologists that clinical pathology is a legitimate special branch of medicine and those physicians who limit their work to clinical pathology are subject to the same code of ethics that governs all other physicians and that a copy of these resolutions be respectfully submitted to the American Medical Association. The resolution was passed with an amendment that the attention of the American Medical Association be called to the fact that they are soliciting advertising from clinical pathologists.

A letter from the Council on Medical Education and Hospitals of the American Medical Association was read by Secretary H J Corper inviting the American Society of Clinical Pathologists to be represented at their annual conference in February each year in the special conference on the subject of private clinical laboratories. A motion was made, seconded, and carried that the President of the Society be empowered to appoint three representatives of the Society to accept this invitation.

Secretary H J Corper read a letter from Mrs Erna Burdick and Miss Georgiana Burdick expressing their appreciation of the institution of the Ward Burdick Research Award of the American Society of Clinical Pathologists.

The proposed amendments to the constitution and bylaws were read and adopted as follows:

ARTICLE III--MEMBERSHIP

SECTION 3

Associate members shall be graduates of recognized scientific institutions who have made such contributions to any of the sciences relating to clinical pathology and whose membership will so further the objects of the Society as to make them eligible for associate membership. Associate members shall pay the regular dues and have all the privileges of Fellows except those of voting and holding office.

Active members shall be designated as Fellows wherever that wording occurs as follows: Article III Section 1 line 2; Section 2 line 1; Article IV Section 3 line 2; Section 4 line 2; Article VI line 2. By Laws Article I line 1; Article II Section 1, line 1; Section 2 line 4; Article III Section 1 line 1; Section 2 line 1; Article IV, Section 3, line 2; Article V line 1; Article VII Section 1 lines 5 and 6.

ARTICLE IV--OFFICERS

SECTION 1

The officers of this society shall consist of a President, President Elect, Vice President, Secretary, Treasurer, Executive Committee, Board of Censors, and Board of Registry of Technicians.

SECTION 5

The Board of Registry of Technicians shall be composed of six Fellows of the Society who shall each hold office for three years or until their successors are elected, two to be elected annually. The first Board shall consist of six Fellows, two of whom shall be elected for a term of one year, two for a term of two years and two for a term of three years. It shall elect its own Chairman from among the holdover members and Secretary and Treasurer.

SECTION 6

Vacancies in the interim on the Executive Committee, Board of Censors, or the Board of Registry of Technicians shall be filled by appointment by the President.

ARTICLE V--DUTIES OF OFFICERS

SECTION 6

The Board of Registry of Technicians shall conduct a Registry of Technicians, receive applications for such, pass on their qualifications and issue certificates to those meeting the requirements. They shall investigate schools for the training of technicians registering those approved. They shall conduct a placement bureau for technicians.

A resolution was passed to extend a vote of thanks to the Local Committee on Arrangements for their good work in assuring the success of the Convention.

The election of officers for the ensuing year resulted as follows: President, Dr. J. H. Black, Dallas, Texas; President Elect, Dr. Kenneth M. Lynch, Charleston, S. C.; Vice President, Dr. H. H. Foskett, Portland, Oregon; Secretary and Treasurer, Dr. H. J. Corper, Denver, Colorado; Executive Committee, Dr. F. W. Hartman, Detroit, Michigan, three years, and Dr. Alfred S. Giordano, South Bend, Indiana, three years; Board of Censors, Dr. Frederick H. Lamb, Davenport, Iowa, three years; Dr. Warren T. Vaughan, Richmond, Va., three years; Board of Registry of Technicians, Dr. Philip Hillkowitz, Denver, Colorado; Dr. Kano Ikeda, St. Paul, Minnesota, three years; Dr. Alvin G. Foord, Buffalo, N. Y.; Dr. C. Y. White, Philadelphia, Pa., two years; Dr. E. S. Maxwell, Lexington, Ky.; Dr. William M. Thalheimer, Chicago, Illinois, one year.

The new President, Dr. J. H. Black, was inducted into office. Meeting adjourned.

The Journal of Laboratory and Clinical Medicine

VOL. XV

ST. LOUIS, MO., JANUARY, 1930

No. 4

Editor WARREN T. VAUGHAN, M.D.

Richmond, Va.

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Official Organ of the American Society of Clinical Pathologists and the
American Association for the Study of Allergy

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EDITORIALS

Multiple Malignant Neoplasms

MULTIPLE malignant tumors excite curiosity and stimulate interest in neoplasms. Basal cell epitheliomas are the most common of the multiple primary newgrowths. Owen, in 1921, found that next in order of frequency among multiple newgrowths, were combinations of basal cell and squamous cell carcinoma of the skin, and then, in succession, followed multiple squamous cell epitheliomas of the skin, multiple carcinomas of the breast, then carcinoma of the breast associated with some other type of carcinoma in some other organ.

The skin is the most common site for the development of multiple malignant neoplasms. Majoi, in 1918, observed that, disregarding the skin, multiple primary malignant tumors do not have a predilection for organs of the same system, except for paired organs, which is in contrast to their predilection for single organs. He observed that malignant newgrowths, again disregarding the skin, were more common in unrelated organs. When two related tumors are found in the same organ, one must answer the question: Is

this an example of metaplasia or are there two distinct primary tumors? Similar multiple malignant tumors in the same organ may be caused, for example, by carcinoma arising from several centers simultaneously or they may be merely a coincidence. Patients in whom multiple malignant tumors develop apparently have a neoplastic tendency which results in these numerous tumors.

Some of the peculiar features of multiple malignant neoplasms should be appreciated by clinicians, by pathologists and by surgeons. Application of such knowledge to the treatment of patients who present themselves with multiple malignant tumors will react to the advantage of patients and to the credit of those on whom responsibility for their treatment is imposed.

—H D Caylor

Erratum

Referring to the article, 'An Exploration Electrode to Determine the Hydrogen Ion Concentration of Fluids in Living Tissue,' pp 181-4 of the November 1929, issue of this journal certain essential data were not included and the author has submitted the following supplementary notes to be considered in connection with the original article.

The parts A, B and C in Fig 1 of that article are $\frac{2}{3}$ actual size and those in Fig 2 are $1\frac{1}{2}$ times actual size. The electrode wires must be 10-11 mm long and not less than but not much more than 1 mm thick. They must be of a platinum alloy rich in iridium and the wire should be polished until smooth. Platinum-iridium electrodes are especially useful and reliable under trying conditions where electrodes made of pure gold or of platinum give variable and low readings.

This electrode is giving satisfactory results in our laboratory. It allows the determination of hydrogen ion concentrations of viscous fluids or solutions containing sediments and coarse suspended matter which would clog a foil electrode. Often even the P_H of a fluid which would poison a foil electrode can be obtained. The arrangement in Fig 2 gives good results with thick, almost solid gels, by merely placing a drop of freshly boiled pure water at the point of contact. With the foil electrode the solution has to be saturated with hydrogen before equilibrium is reached and with increase in volume the time is proportionately longer. The correct EMF with the point electrode is almost always obtained instantaneously regardless of the amount of fluid used. With some solutions, especially those low in buffer the EMF has a tendency to drop and is highest only at the first moment of contact. On the other hand, with strongly buffered physiologic solutions the P_H remains constant.

The values obtained with this electrode agree with the values obtained with the foil electrode for the whole P_H range.

The electrode is well adapted for P_H determinations on a large scale. Sixty tests an hour can readily be made with pure buffer solutions. Over five thousand EMF determinations have been made during the past year in this laboratory, where it has supplanted the foil electrode for almost all except electrometric titrations.

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News and Notes

To the Members of the American Society of Clinical Pathologists

By unanimous agreement of the Research Committee, President Black, and members of the Executive Committee, it has been decided to use the subject, "Agranulocytosis," as the topic for the symposium at the Detroit meeting, discussing it chiefly as the condition first described by Schultz as "Agranulocytic Angina," but also including those cases with lesions elsewhere than in the mouth, and also cases with blood pictures showing agranulocytosis along with other features in the blood picture or etiology to exclude the diagnosis of agranulocytic angina of Schultz. The members of the Society are earnestly requested to summarize their findings in cases observed by them in recent years so that the information from all may be presented at the next meeting. To facilitate the reporting of cases, questionnaires will be sent from the Secretary's office some time in the spring of 1930, but meanwhile it is hoped that cases studied in the past and those in the next few months will be completely worked up preparatory to summarizing in the questionnaire.

The united efforts of the membership are also asked for in the study of the question as to the avian tubercle bacillus being the cause of Hodgkin's disease as suggested by Dr L'Esperance from her chicken inoculation experiments which she reported before the last two meetings of the Society of American Pathologists and Bacteriologists. By pooling the results of well controlled experiments conducted by our members throughout the country, some concrete ideas to the rôle played by the avian strain can be formulated.

Further endeavor to uncover undulant fever cases and to carry on the work begun last year appears also to be quite in order and again we hope to accumulate more data from our members in all the types of laboratories served by them. Please keep this subject in mind as one of the problems to be studied as a joint effort of the Society.

Trusting that you can contribute personally to all of the three problems, we remain
Fraternally yours,

Research Committee,
ALVIN G FOORD, Chairman,
Buffalo General Hospital, Buffalo, New York

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No 5

AMERICAN SOCIETY of CLINICAL PATHOLOGISTS

BRUCELLA ABORTUS INFECTION IN MAN*

A CLINICAL ANALYSIS OF THIRTY FIVE CASES

BY ALFRED S. GIORDANO, M.D., M.Sc., AND R. LLOYD SENSENICH, M.D.
SOUTH BEND, INDIANA

DURING the past three years there has been reported the widespread existence of a disease caused by infection with organisms belonging to the group of *Brucella alkaligenes*. In this group there are several strains, classified according to the animal from which they were originally isolated: the caprine, bovine, porcine, and others. The caprine strain, *Brucella melitensis*, was first isolated and described by Bruce¹ in 1887. We are indebted to Hughes for a classical clinical description of the disease resulting from this infection, which is characterized by attacks of undulatory pyrexial relapses, profuse sweats, rheumatic pain, and enlarged spleen.

Another strain now known as *Brucella abortus* was isolated by Bang² in 1897 from the placentas of aborting cattle, but it was not thought to be pathogenic for man. Evans³ later showed the similarity of the cultural and serologic characteristics of *Brucella melitensis* and *Brucella abortus*, and suggested that *Brucella abortus*, like *Brucella melitensis*, might cause disease in man. This theory was first definitely proved in 1924 by Carpenter,⁴ whose experiments fulfilled Koch's postulates. Since his publication many cases of *Brucella abortus* infection in man have been reported. Confusion has, however, arisen in that the relationship between *Brucella abortus* and *Brucella melitensis* has apparently led to the assumption that *Brucella abortus* infection in man must necessarily follow a clinical course identical with that produced by infection with *Brucella melitensis*.

Read before the Eighth Annual Convention of the American Society of Clinical Pathologists, Portland, Oregon, July 5, 6, and 8, 1929.
From the South Bend Medical Laboratory.

In 1927, while studying the clinical features of *Brucella abortus* infection, we⁶ presented a preliminary report of a series of seven cases. These patients were under observation for a relatively short period. No attempt was made at the time to classify the cases studied except along the lines generally followed in the consideration of cases of undulant fever due to *Brucella melitensis* infection.

With opportunity for prolonged observation and the addition of twenty-eight cases to the group studied, the relative infrequency of the typically undulant febrile course raises the question as to whether or not *Brucella abortus* infection in man tends to follow a clinical course somewhat at variance with that of the disease due to *Brucella melitensis*. On the basis of additional information gained by more complete study, these variations may now be more definitely described.

INCIDENCE

Sex does not seem to be a factor. In our series there are 20 men and 15 women. The incidence by decades is as follows:

AGE, YEARS	CASES
11 to 20	1
21 to 30	9
31 to 40	13
41 to 50	9
51 to 60	2
61 to 70	1
Total	<u>35</u>

In the total of 35 cases there is a preponderance of infection in the second, third, and fourth decades. The youngest patient is sixteen and the oldest sixty-one years of age. The reports in the literature record no cases in children below eight years of age. This apparent immunity in children parallels that observed in young calves.

Occupation is an etiologic factor only in so far as it provides opportunities for infection. Bacteriologists working with these organisms, veterinary surgeons or others engaged in animal husbandry, or those handling the carcasses of infected animals. The incidence of infection in this class is extremely low as compared with those in which infection is traceable to the ingestion of infected milk.

Hardy⁷ recently reported an analysis of 125 cases in which 82 patients were engaged in occupations in more or less direct contact with live stock, meats, or dairy products. Among these, 56 were farmers, 8 farmers' wives, 3 stock buyers, 12 packing-house workers, two workers in dairy products, and one a butcher. The remaining 43 were distributed among various occupations and professions. From this analysis the only direct occupational incidence is that of the 12 packing-house employees, and in this group it would have to be proved that raw milk had not been ingested. Certainly one could not classify the incidence among farmers as an occupational disease, unless aborting animals were treated. The high incidence in this class must also be considered from the basis of the consumption of infected milk, just

as among urban dwellers with no contact with infected animals in communities where pasteurization of milk is not practiced

In our series, 5 patients were farmers one a veterinary surgeon, one a mechanic who also raised pigs and on occasion handled infected placentas and the remaining 28 were in urban occupations such as trades and professions including three physicians. In only one case in this series was contact infection established this patient was a veterinary surgeon

RESIDENCE

The incidence of the disease in small communities is usually much greater as there are fewer pasteurization plants, and raw milk is more generally consumed. Of the 35 cases herein reported only six patients lived in towns of more than 50,000. The remaining cases occurred in small communities of less than 20,000, and many of these patients visited relatives owning farms whose herds were found to be infected with *Brucella abortus*. The importance of *Brucella abortus* infection as a rural health problem is at once apparent since in all of our cases the patients consumed raw milk.

MODES OF INFECTION

While the modes of infection are variable occurrence of the disease following the ingestion of milk containing *Brucella abortus* in cases reported by us Carpenter,⁸ Huddleson,⁹ Evans,⁶ Kern,¹⁰ Simpson,¹¹ and others leaves no doubt as to the manner of infection in these cases.

INCUBATION PERIOD

In only one of our cases could the incubation period be approximately determined. In accidental laboratory infections the incubation periods have varied from seven to twelve days.

MODE OF ONSET

The initial symptoms are as widely variant as is the clinical course. An insidious onset with fatigue, anorexia, low fever, headache with muscular weakness or joint discomfort is the most common. Chills are usually not the initial symptoms and develop after elevation of temperature is noted. The presence of pharyngitis, slight cough or bronchitis frequently lead to a mistaken diagnosis. Rarely is the onset more abrupt and the evidences of sepsis are the first and temporarily overwhelming symptoms. One such patient ran a very brief course with early recovery and without relapse whereas a rapid malignant course with hyperpyrexia and fatal termination has been reported by Hardy⁷ and others.

Arthritis with moderate elevation of temperature may be the first and only manifestation of the disease or the nervous system may be attacked and the invasion may be characterized by meningeal symptoms or neuritis.

CLINICAL FEATURES

The disease may be acute or chronic the course in each instance differing not only in duration of illness but in other respects and varying to such a degree as to be distinctive. One of our patients with chills, sweats, headache

and fever of septic type, with no tendency to undulation, recovered in two weeks with no subsequent relapse, whereas another patient has for a period of illness of more than ten months had a continual course of chills, sweats, and septic type of fever without undulation or a single remission. In a third case, reported in an earlier communication, the patient suffered an illness of six months' duration characterized throughout by undulating temperature and apyrexial remissions.

The chronic course of the disease has been commonly described in the literature of undulant fever, whereas the acute course has been infrequently discussed, or discussed with the assumption that the apparent short course was accounted for because of an insufficient period of observation and that the illness must have included many such attacks with periods of remission.

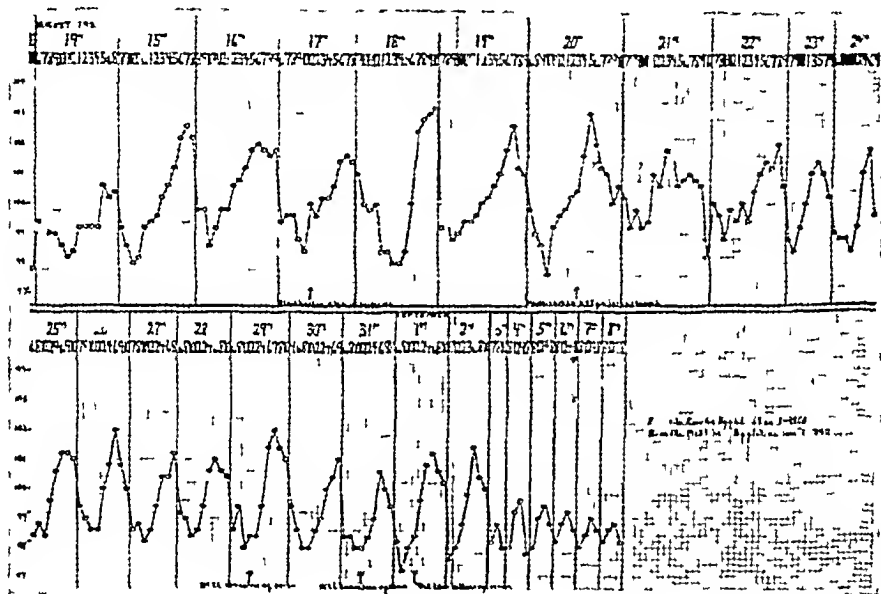


Fig 1

As a result of this, the chronic type has been generally accepted as the most frequent and typical. From our observations in *Brucella abortus* infection and a study of those cases reported in the literature, it is apparent that the cases of acute type are more frequent than the chronic type described, and it is suggested that the tendency to acute or chronic illness may be a differential point between the *Brucella abortus* and *Brucella melitensis* infection.

Although the disease due to *Brucella abortus* presents a widely varied symptomatology and although certain symptoms may increase or diminish in intensity or may be supplanted by other symptoms, there is noted in the cause of illness in most cases a dominance of certain symptoms directly referable to the particular body structures that are being attacked by the disease. For purposes of better comparison and study, therefore, an effort will be made to define more closely, and to classify, cases of this disease on the basis of predominant symptoms and various body tissues attacked. That this classification may be practical and helpful, we have considered under the following

subdivisions all the cases that have come to our attention. The brief histories submitted were chosen as examples typical for the group.

Septic Type—Into this group we have placed those cases in which the course of the disease has been similar to other infectious diseases in which bacteremia is an underlying condition.

A man, aged thirty-two, a physician, became ill on July 1, 1928. The onset of the disease was acute with chills, fever, profuse perspiration, marked weakness and rapid loss of weight (15 pounds). Headache and backache were distressing and there was pain in both eyes. A slight cough persisted throughout the first ten days of the illness. There was an emotional instability markedly contrary to the man's usual personality. The fever was septic in type reaching 102.6 to 103° F at six o'clock in the evening and exhibiting marked morning remissions. There was no tendency to undulation. Physical examination revealed no abnormalities aside from an old mitral valvular lesion and tenderness of both testes which developed soon after the onset of this illness. The urins were normal. The hemoglobin was 90 per cent, erythrocytes normal, leucocytes 4,300 polymorphonuclears 42 per cent, small lymphocytes 47 per cent, large lymphocytes 10 per cent, eosinophiles 1 per cent. Agglutination tests for *Bacillus typhosus* and *Bacillus paratyphosus* A and B were negative. *Brucella abortus* agglutinated in a dilution of 1:280 and *Brucella melitensis* in a

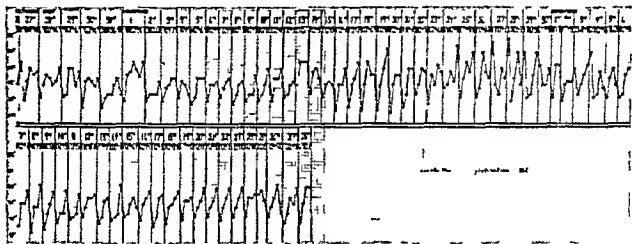


Fig. 1

dilution of 1:160. The intradermal test was positive although blood cultures remained sterile. The patient was treated with salvarsan without effect. Convalescent serum was given with no immediate results, although after two weeks of this treatment the temperature gradually became normal. Recovery was complete in three months and there has been no relapse.

Arthritic Type—Into this group we have placed those cases in which dominant symptoms have been referable to the joints. They have varied from painful tender joints in which the involvement may have been periarticular to conditions in which recurrent hydrops was the characteristic manifestation.

A well-nourished boy, aged sixteen, in July 1926, complained of "rheumatism in both knees" and of profuse sweats. In October, 1926, following an injury, an abscess developed in the right leg below the knee. This was drained surgically and gave no evidences of association with the joint symptoms described. There was no evidence of bone involvement and x-ray examination was negative. Indefinite joint symptoms continued until February, 1927, at which time he suffered an attack of arthritis involving the shoulders, elbows, wrists, hips, knees, ankles and feet. This attack was of short duration but recurrent shifting joint symptoms continued. The attacks were accompanied by fever ranging from 99.5 to 104° F, with chills, profuse sweats, a furred tongue and some tenderness to pressure over the liver. Physical examination in the interim between attacks did not reveal any abnormal

and fever of septic type, with no tendency to undulation, recovered in two weeks with no subsequent relapse, whereas another patient has for a period of illness of more than ten months had a continual course of chills, sweats, and septic type of fever without undulation or a single remission. In a third case, reported in an earlier communication, the patient suffered an illness of six months' duration characterized throughout by undulating temperature and apyrexial remissions.

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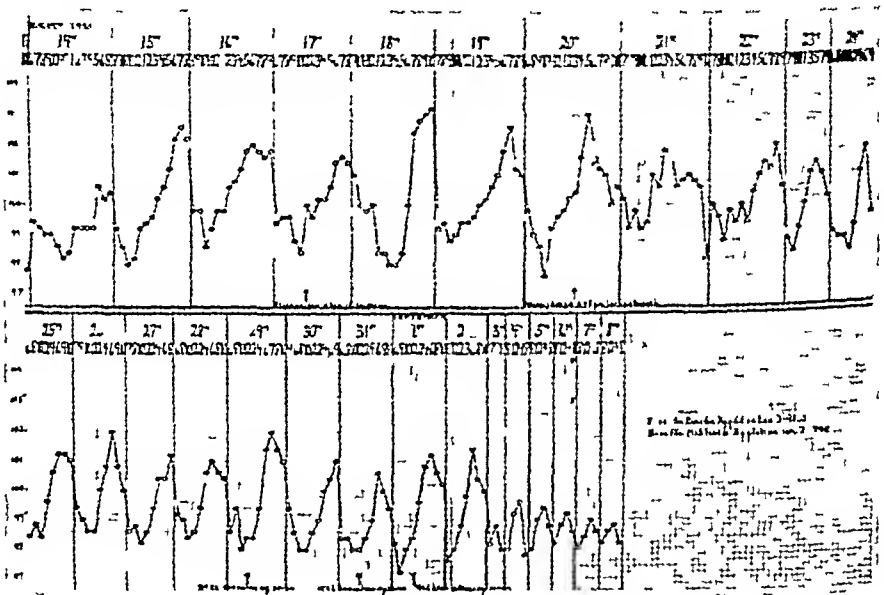


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Visceral Type—The bacteremia in this disease leads to visceral invasion, and lungs, heart, liver, gall bladder, bowel, spleen, kidneys, uterus and adrenals have been involved. Abortions have occurred in women infected with *Brucella abortus*, although we have seen no reports of abortions due to *Brucella melitensis*. Under this visceral type we have grouped all patients having dominant visceral symptoms.

a. An automobile dealer forty years of age, a patient of Dr. J. C. Fleming, came under observation on April 24, 1928. His history contained nothing of moment other than an attack of painless jaundice three years prior to this illness. At that time the gall bladder had been drained and improvement had followed. The patient had remained in good health until March 28, 1928, when he noted a loss of appetite, fever and jaundice. The fever was septic in type and gradually rose until it reached 104° F. At this time tenderness over the gall bladder was noted. The surgeon considered cholecystectomy inadvisable at the time so a cholecystostomy was done. The leucocyte count was 5,000. Agglutination of *Bacillus*

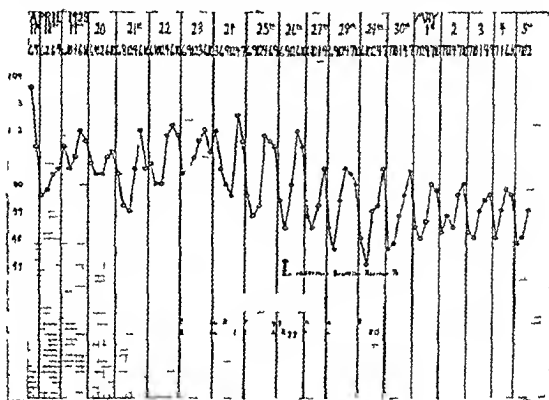


Fig. 4

typhosus occurred in a dilution of 1:10. Agglutination of *Brucella abortus* occurred in a dilution of 1:1,800 and *Brucella melitensis* 1:80. Culture from the bile was made and the organisms injected into two guinea pigs and they became infected with *Brucella abortus*. The intradermal skin test was positive for *Brucella abortus*. There was no immediate improvement after operation. Fever and sweats continued for two weeks after which gradual recovery took place. Jaundice disappeared after cessation of fever. The course of this illness was of about two and a half months duration. There has been no recurrence.

b. The brief history available in this case is strongly suggestive. The patient, a woman who had been married for ten years, had always been in good health prior to the present illness. In the first six years following her marriage she had given birth to five children. All pregnancies had been normal and labors normal and uncomplicated. All of the children are living and well. During the past four years she has suffered from marked weakness and pain in the back and legs. Headaches have been so severe that it has been almost impossible for her to be up and around. The patient has had frequent attacks of chills and fever which have persisted for periods of two or three months. During this period of four years she aborted four times at about the fifth month of pregnancy. History and physical examination failed to reveal any cause for the repeated abortions. The urine was normal.

Blood examinations for malaria and the Wassermann reaction were negative. *Brucella abortus* agglutinated in a dilution of 1:160.

c. A man aged thirty-five, a physician, previously in good health, became ill in November, 1928, with fever, chills, sweats, backache, epigastric pain and vomiting. There was marked muscular weakness and loss of weight. The temperature was remittent and continued for four months. On physical examination the liver was found to be markedly enlarged and tender. There was no jaundice. The heart and lungs were normal. There was no evidence of genitourinary disease or orchitis. The urine was normal. The erythrocytes were normal in number, and leucocytes numbered 6,000 with a normal differential count. Agglutination tests for *Bacillus typhosus* and *Bacillus paratyphosus* A and B were negative. *Brucella abortus* agglutinated in a dilution of 1:1,280. The enlargement of the liver continued for more than four months. Recovery followed at the end of five months and there has been no relapse. The patient states that he has had no residual asthenia or any other symptoms.

Glandular Type—This type is undoubtedly less common than those before described, and includes those cases in which regional glands have been infected by reason of proximity to some point into which a large number of virulent organisms have gained entrance.

A man aged forty, a veterinary surgeon, presented nothing of importance in his history other than acute arthritis at the age of twenty-two. This arthritis had been confined

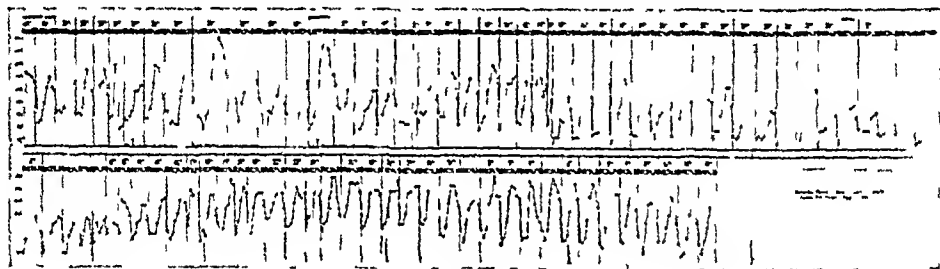


Fig 5

to the feet, knees and hips. There had been occasional recurrent attacks, though not severe or disabling. He was then a student in the department of agriculture of a large university and was specializing in animal husbandry. He later completed a course in veterinary surgery and in 1925 came under observation because of enlarged tender and painful axillary glands, and attacks of chills, fever, sweats and backache. The attacks of fever, chills and sweats were always an accompaniment of the swollen axillary glands and in periods of recession of the glandular inflammation the acute symptoms were absent. Backache and general malaise, however, frequently continued for long periods and the axillary glands were palpable. Hospital observation and laboratory examination failed at that time to reveal the character of the infection. Following a period of rest the patient's condition was markedly improved. In May, 1928, after a period of unusually distressing backache, agglutination tests were made. *Brucella abortus* did not agglutinate. The intradermal test was positive for both *Brucella melitensis* and *Brucella abortus*. More recently there has been an acute attack with chills, high fever, sweats and extremely distressing headache and backache. The axillary glands were again markedly swollen, painful and tender to pressure. As a veterinary surgeon the patient is often called on to care for aborting animals. In this work he has frequently acquired infections on the hands and arms, and has recently observed that the exposure of the unbroken skin of his arm to abortus-infected animal discharges will cause the appearance of an eruption and if the skin is not carefully cleansed there may be a recurrence of glandular swelling and acute symptoms. Considering the university course in animal husbandry, it is impossible to determine the date of the original infection or number of possible reinfections because of the frequent exposure to infected animals throughout a period of nearly twenty years.

SYMPTOMS THEIR FREQUENCY AND CHARACTER REGARDLESS OF TYPE

Fever—Of the individual symptoms, fever is the most constantly present and the most variable in character. Owing to the bacteremia of this disease, the fever in most cases is somewhat similar to that associated with other septic conditions. The undulant type of fever is the most striking but more often a feature of the chronic cases. The temperature for a period of from seven to fourteen days rises by step like gradations, each day reaching a higher point and exhibiting a morning remission to or near normal (Fig 6). The fever having reached a daily peak of 103° or 104° F with a morning remission of two or three degrees, begins to exhibit the undulatory phenomena as follows. The daily peak and low point in remission each day occur at a higher point than the preceding day until the crest of the wave is outlined whereupon for an equal number of days the temperature in like manner recedes to a lower level each day. Such a temperature chart viewed over a period of weeks exhibits a series of undulations each requiring the same number of days for completion.

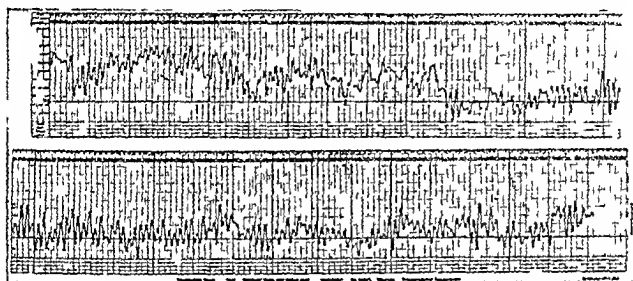


Fig 6

In one of our cases the undulations covered eight days, while in another one the cycle was completed in four days. In the melitensis infection each cycle may cover a period of three weeks or more. Hughes,² who first suggested undulant fever in *Brucella melitensis* infection as descriptive of this phenomenon noted the variable length of the undulations in different cases but pointed out the striking constancy of the undulatory pattern once it was established in a given individual. The period of typical undulation is variable in length. In one of our cases it existed for approximately four weeks while in another this type of fever persisted for many months. There is a gradual tendency to lower levels with apyrexial intermissions followed by short periods of relapse with irregular fever and change to the intermittent type. This in one of our cases persisted for a period of six months. This undulant febrile record is parallel to that of undulant fever due to *Brucella melitensis*. Such a case was reported in our first series. With opportunity for study of temperature records of many more patients, we are now rarely finding this typical febrile pattern. We are seeing instead a tendency to constant septic fever with moderate daily remissions, variable in course from a period of two weeks to ten-

months, without tendency to undulation or apyrexial intervals. This, too, has recently been pointed out by Hardy.⁷

It is also noted that after improvement has begun there is steady and fairly rapid progress to an apparently complete restoration of health without sequelae or recurrence. Inasmuch as our studies are of *Brucella abortus* infections and our findings are apparently at variance with the more frequently reported undulant febrile course in *Brucella melitensis* and although mild and intermittent types of that disease are also reported, it is suggested that the *Brucella abortus* infection may tend to the production of an illness of shorter duration, at the same time differing from the *melitensis* infection in febrile course. In view of the difficulty in diagnosis of the short acute illness, it is probable that with an awakened interest on the part of physicians and increased facilities for laboratory diagnostic tests for this disease, that a still larger proportion of cases running a short course will be discovered.

Chills—Chills are equally as constant as is fever. They are present early in the illness and tend to persist, although they occur less frequently later. They vary in intensity from a mild chilliness to violent rigor and are usually followed by a rise in temperature, but may occur only as a distressing chilliness while the temperature is elevated. Recurrence at the same time daily throughout a long period of time is frequently noted.

Sweats—Sweats are associated with the periods of chills and hyperpyrexia. They may be mild or extremely profuse and may occur independently of chills. These sweats may be of noticeably offensive odor.

Headache—Headache, especially in the early stages of the illness, is constant and extremely distressing. It is more frequently occipital and the pain extends downward through the cervical region and then is diffused over the shoulders and upper portion of the back. The severe pain has a tendency to regular periodicity, recurring at the same time each day, usually with increases in temperature. Later in the illness and in the presence of lower temperature, it is lessened or disappears.

Backache—Backache is at first usually related to the occipital headache as before described, and generally diminishes in intensity with it. It may, however, persist independently or even recur after the febrile period. It is frequently confined to the lumbar region.

Arthralgia—Arthralgia is fairly constant and the onset is usually early in the illness. It is generally lessened with disappearance of the acute septic symptoms. It may, however, persist throughout the illness. It is variable from a mild discomfort to a persistent pain in the smaller joints likened to the pain of a crushing injury. Less frequently is there discomfort about the larger joints. The pain is apparently due to periarticular neuritis.

Arthritis—Arthritis is a distressing and frequently disabling manifestation of this disease. It may develop early or late in the course, or may exist as the dominant symptom throughout. In some cases it has apparently been the only manifestation of the disease. All the joints may be involved, separately or together, or it may shift rapidly from joint to joint. There may be a definite periodicity of return as in one of our cases, in which acute attacks recurred at regular intervals for many months. The joints involved may be

painful and tender, but even though they are swollen there is seldom redness. There may be marked hydrops which recurs at definite intervals of days or weeks, develops rapidly and recedes promptly as in a case reported by us and one reported by Baker¹. In our case cultures of the organism were obtained from fluid aspirated from the involved joint. No permanent impairment of joint function followed.

Neuritis—Neuritis, like arthritis, may occur at any stage of the illness, and may be of variable duration or the only symptom of the disease. It may be evidenced by pain in any locality and is probably responsible for most of the pain about joints and in the back, as was described. There may be a noticeable cutaneous hyperesthesia, but no areas of anesthesia have been demonstrated in our cases. An unusual type of discomfort is that described by many of our patients as "eye ache." It is not due to photophobia, is not caused or accompanied by visual disorders, and may be present at any time. Persistent or recurring sciatia is frequently due to this infection.

Other nervous manifestations of this disease are present almost without exception. Asthenia is an early and constant symptom and frequently persists for a long time after relief from all other symptoms. Depression, irritability or anxiety, neurosis are frequent variations in the mental condition of the patient. Even in the presence of high fever there was never the dullness or apathy, such as commonly characterizes the typhoid state. Exaggerated reflexes and evidence of tension are common. Tremor occurs and in one of our patients the whole body was affected. This was so pronounced that intelligible speech was almost impossible. A complete recovery followed. Insomnia is frequently a troublesome symptom. Psychic and functional nervous disorders are the most common sequelae.

Gastrointestinal Symptoms—Anorexia is frequently the first symptom noted, but there is usually a return of appetite as the acute febrile stage subsides. Aside from anorexia and constipation gastrointestinal symptoms are not common. Nausea and vomiting occur rarely. Epigastric distress is an occasional symptom. In two cases cholecystitis was suspected, and in one instance the gall bladder was drained but without relief of symptoms. There is nothing distinctive about the appearance of the tongue.

Orchitis and Oophoritis—Orchitis and oophoritis are frequently present. They usually occur during the febrile periods of the disease. Orchitis was a persistent and distressing symptom in two of our cases in which the clinical course had been most mild. There is tenderness and perhaps some swelling. In our cases there was no history of previous disease. This symptom may persist after recovery from other acute symptoms.

Skin Manifestations—Unusual skin lesions were seen in six of our patients. In two instances these occurred as tubercle-like elevations on the dorsal surface of the fingers and hands. In others it also involved the face and body. They appeared as small maculae and persisted for a few days. In one patient these macular lesions were accompanied by an intense itching that lasted for two weeks.

Splenic Symptoms—The spleen was palpable and tender in two of the cases in our series. Notable enlargement of the spleen so commonly observed

in *Brucella melitensis* infection as to be regarded as a cardinal symptom, was observed only once and that was in a patient whose blood agglutinated *melitensis* only

Hepatic Symptoms—Definite enlargement of the liver occurred only twice in our series. In one case it was associated with vomiting. In three cases under our observation there were symptoms suggestive of gall bladder disease, and in one case there was jaundice. A cholecystostomy was done on the jaundiced patient and in this case *Brucella abortus* was obtained on culture from the bile. The jaundice continued after the operation and until the febrile stage had passed.

Renal Symptoms—The kidneys may be impaired. A moderate amount of albumin and a few casts were found in some of our cases, although considering the character of the illness such changes were remarkably rare. The organism was not found in urine cultures.

Respiratory Symptoms—Pharyngitis is frequently an initial complaint. Pulmonary symptoms are common. Bronchitis varying in intensity may come early in the disease and persist throughout. Pneumonia and pleurisy with effusion may occur. Diagnosis of pulmonary tuberculosis was made in one of our cases.

Cardiac Symptoms—Heart manifestations due to *Brucella abortus* are fortunately not common. The pulse rate is accelerated and in the absence of fever the case might be mistaken for one of hyperthyroidism. Rhythm and valve sounds are not often altered unless this occurs in previous disease. However, one death from endocarditis due to this organism has been reported by Saphir¹³

Affection of Lymphatic Glands—Lymphatic gland involvement is usually not demonstrable in this disease, except in the cases of regional glands which have become infected by reason of close proximity to the point of introduction of an overwhelming number of virulent organisms. This condition exists in one of our patients, a veterinary surgeon, whose axillary glands are enlarged and tender, due probably to repeated infection from operative wounds sustained in handling infected cattle. The demonstrable changes in these glands vary with the various stages of activity of his infection as evidenced by clinical phenomena and blood examination. No doubt infected lymph glands frequently harbor this organism throughout protracted periods. Carpenter has repeatedly cultured organisms from tonsils removed at operation.

DIAGNOSIS

Undulant fever as regards diagnosis on the basis of clinical observation alone requires differentiation from many lesions due to infection. Typhoid fever, malaria, tuberculosis or other respiratory infections, and sinusitis, rheumatism, cholecystitis or liver abscess, meningitis or any pyogenic infection must be considered. The recent report of cases of leishmaniasis in this country might rarely make it necessary to consider the possibility of this disease. The differential diagnosis from each of the above-named conditions cannot be discussed in detail in this paper.

The history as to probable ingestion of infected milk or contact with infected animals is frequently strongly suggestive. The chills, fever, sweats, muscle or joint discomfort, headache and evidence of involvement of the nervous system without gastrointestinal symptoms, rose spots, hemorrhage or demonstrable focal infection furnish valuable differential evidence although all these variations may occur. A persistently chronic course uninfluenced by quinine or salicylates adds therapeutic evidence if no laboratory facilities are available. Definite diagnosis must be made by the clinical pathologist.

CLINICAL PATHOLOGY

The leucocyte count early in the disease is usually diminished. The lowest count was 3,800 and the highest was 14,000. Later in the course the count varies from 6,000 to 10,000. The differential count is usually normal or there may be a slight increase in mononuclears but this too is a variable factor. The erythrocyte count and the hemoglobin have always been well within normal limits. The urine is essentially negative. Blood cultures have been extremely difficult to obtain in this series but of twenty cultures only two were demonstrated as positive. On the second attempt in one case a few colonies were grown on Huddleson's liver agar media in 10 per cent CO₂ atmosphere, although the guinea pig inoculations were negative. In another case the guinea pig inoculation was positive, and the culture negative. It appears that positive cultures are more likely to be obtained at the height of temperature.

AGGLUTINATION TEST

There is no dispute that the agglutination reaction is the most valuable sign in the diagnosis of undulant fever. In the majority of instances agglutinins appear early in the course of the disease. There are several reports in the literature of patients with no agglutinins in the blood serum, but with positive blood cultures. Tramontano in 65 confirmed cases of undulant fever found nine serums which failed to agglutinate. Carpenter¹⁴ isolated the organism in five cases of undulant fever, in only three of which agglutinins were present. Our cases suggest this possibility but further investigation of this point is indicated.

The agglutinin titer varies from 1:5 to 1:20,000. Serums agglutinating *Brucella melitensis* or *Brucella abortus* in a dilution of 1:40 or higher, it is generally agreed, indicate active infection with these organisms but the significance of agglutination in low titer is still open to discussion. It has been suggested that absorption of agglutinins from ingested milk may take place. This seems a little difficult to understand in view of the original low agglutinin content of milk. It is also known that absorbed agglutinins are excreted rapidly, yet in our experience some patients with agglutinins in the blood had not consumed milk for months previous to the test. Others regard these agglutinations as nonspecific. Evans believes the agglutinins arise as a specific response to infection with *Brucella abortus* ingested with cow's milk although such an infection may not result in illness. A study of our series seems to substantiate this conclusion.

Agglutinins may result from an old infection from which the patient has entirely recovered. They may be present in the blood of a carrier. This assumes a focus of infection and constant agglutinin production to combat the organisms released, or it may be the response to a recent active infection. Instances of the carrier state have been cited by Shaw¹⁵. He was able to isolate *Brucella melitensis* from the blood of ten Maltese dockyard employees, none of whom exhibited symptoms. Vaccaro also isolated the organism from the urine of a patient who was apparently well but whose blood contained agglutinins for *Brucella melitensis*.

In the present study most of the serums from actively ill patients agglutinated in dilutions higher than 1:160. On the other hand, in two active cases the serum did not agglutinate in more than 1:10 dilution. In general it can be stated that the agglutinin bears no relation to the activity of the illness and the agglutinins remain in the blood for more than ten years. Our own study of this problem has convinced us of the necessity of using several strains of this organism in the routine test. One patient who gave a history of at least three years of recurring attacks of fever, chills, headache, and excruciating lumbar pain, had been under medical supervision for a long period. Preliminary agglutination with *Brucella abortus* (No. 80, Meyer) was negative. Because of the dominant symptoms of this disease, seven other strains were used, including one *paramelitensis* and two other *melitensis* strains of ascertained origin. This subsequently showed complete agglutination of *Brucella melitensis* in dilutions up to 1:1,800 with all *melitensis* strains. Another complication that is occasionally encountered is the so-called zone phenomena or preagglutination zone in which agglutinations occur only in high dilutions. Cross agglutinations with *Bacterium tularensis* were reported recently by Francis and Evans,¹⁶ but the difference in the titer clears the diagnosis.

AGGLUTINATION TITER AND PROGNOSIS

On the basis of the present data, as has already been stated, there is no relationship between the titer of agglutinins and the severity of the infection. Usually, however, the titer gradually falls as the patient recovers, but a proportionately high titer is maintained for at least one year or more.

IMMUNITY

At present there are no available data on the subject of immunity, but with present knowledge one might infer that natural immunity is present in many cases.

DERMAL TEST

With the recognition of serologically negative cases of *Brucella abortus* infection an attempt has been made to develop a skin reaction test. As previously reported,¹⁷ this test yields specific skin reactions in known positive and negative cases, and promises to be an additional aid in the diagnosis. With this point in view, the test is worthy of further investigation over a larger series of cases.

TREATMENT

Rest in bed with symptomatic treatment during the period of acute manifestations is the treatment indicated, and has in our series given results equal to any specific therapy thus far advanced. Rest should be maintained until the temperature has continued normal for at least a week and even then relapse may follow physical exercise. Five patients in our series resumed their usual duties without apparent ill effect after a few days in bed. Chemotherapy is suggested as a possible therapeutic procedure, but in view of the low mortality in this disease it should be determined in advance that the administration of a given chemical agent is not attended by undue risk. Reports from the use of mercuriochrome are conflicting and its use does not seem to be justified on the basis of present information. Neutral acriflavin was administered to one patient in the third week of illness and a severe reaction immediately occurred. Disappearance of fever followed in a few days although a relapse occurred after eight months of apparently normal health. Administration of this preparation to another patient had to be discontinued because of the violent reaction when only 5 cc of a 1 per cent solution had been given. No improvement followed.

The efficacy of foreign protein therapy has been reported in the literature. We have used intramuscular injection of milk and intravenous administration of typhoid vaccine but without influencing the course of the disease. Baker¹ has reported beneficial results in a case of intermittent hydrarthrosis treated with convalescent serum but a relapse is said to have occurred later. This treatment was also given by Moss in a case of undulant fever but without permanent results. One of our patients having been treated with salvarsan without results was given convalescent serum. There was no immediate effect but improvement began a week later and continued to complete recovery. Specific vaccine therapy has been used in Europe in treatment of *Brucella melitensis* infections and it seems promising.

There is as yet no general agreement as to results.

SUMMARY

A clinical study of thirty five patients infected with *Brucella abortus* is herein presented in which an effort is made to more closely define the clinical course on the basis of predominance of symptoms as to particular body structures involved.

Results of these observations suggest that *Brucella abortus* infection tends to run a clinical course somewhat at variance from that of *Brucella melitensis* infection.

It is apparent that the illness more frequently runs an acute course septic in type, without characteristic pyrexial undulations or relapses and terminates in recovery without notable sequelae.

The illness, if protracted presents a clinical picture sufficiently distinctive as to make clinical recognition possible but early diagnosis must depend on bacteriologic and serologic studies.

In the light of present knowledge symptomatic treatment with rest in bed is the treatment of choice.

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THE LABORATORY DIAGNOSIS OF UNDULANT FEVER*

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THE recognition during the last five years of the frequent occurrence of undulant fever in the United States has prompted many laboratories to devote considerable time to the study of *Brucella abortus* and *Brucella melitensis* infections in man. There is not much doubt that both organisms now classed as two species originated from one source. Apparently, *Br. abortus* has adapted itself chiefly to cattle while *Br. melitensis* has been able to propagate itself more advantageously among goats. Incidentally, both organisms have become invasive for man and produce symptoms which cannot readily be differentiated. Aside from eliminating the sources of these infections for man, the differentiation is not important, because the diagnosis and treatment, as well as the course of disease caused by the two agents, are the same.

The biologic characters of the two organisms are so similar they can be differentiated only by studying the antibodies produced by each. There may be variations in their atmospheric requirements under artificial cultivation, differences in their degrees of pathogenicity for experimental animals, in their antigenic properties, and in their morphology as well as in their natural habitats, but these cannot always be relied upon. Several investigators have described methods for identifying the two types, but in our experience, with the exception of the agglutinin absorption test, these cannot be depended upon absolutely. Many quantitative differences can be observed when a large number of *melitensis* and *abortus* cultures are examined, but no infallible qualitative test, with the above exception, has been developed. A few cultures of *Br. abortus* are always found that show a characteristic of so called *melitensis* and vice versa.

The agglutinin absorption test requires experience and may fail if employed by one who is not accustomed to the technic or by individuals not familiar with the peculiar cultural characteristics of both species. The test depends upon the use of authentic cultures of *Br. abortus* and of *Br. melitensis* or of antisera prepared from them. We now know that both microorganisms may be isolated from man and that they may be recovered from various species of animals. In our studies we have employed *Br. abortus* "80," isolated from certified milk by Dr. K. F. Meyer¹ and *Br. melitensis*, "428," also obtained by Dr. Meyer from Dr. E. Sergent, Institut Pasteur D'Algerie, Tunis. We know that the antibodies produced by these two cultures are different but are we safe in assuming that they have been named correctly? However, such assumptions must be made because it is necessary to have some foundation upon which future work may be based.

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By the use of the agglutinin absorption test, evidence has been accumulated to show that the majority of cases of undulant fever in the United States are due to *Br abortus*. Because goats have been eliminated as a possible source of infection in most of the reported cases in this country, circumstantial evidence has been presented incriminating cattle, or swine that may have been infected from contact with cattle. It has been well demonstrated that cow's milk is the chief source of infection.²

The cardinal symptoms of undulant fever are general malaise, fever, night sweats, enlarged spleen and perhaps a general lymphadenitis. There usually occurs a slow pulse incompatible with the increase in temperature. If these symptoms continue for many weeks there is a loss of weight and a secondary anemia. The infection may localize in the joints or in the genital tract. Unless one has had considerable clinical experience or does not consider undulant fever in his differential diagnosis, it is readily seen how such symptoms may be ascribed to other infectious agents which produce a similar syndrome. Accumulation of information concerning the presence of undulant fever in this country, as well as a review of the literature on the subject, shows clearly that the majority of diagnoses have been made by those engaged in laboratory work. It is evident that during recent months, the trend has been changing and clinicians are requesting laboratories to confirm their diagnoses.

The various laboratory procedures of assistance in establishing a positive diagnosis of undulant fever are as follows:

- 1 The agglutination test
- 2 The complement-fixation test
- 3 The bacteriologic examination of blood
- 4 The agglutinin absorption test
- 5 The blood count

It is extremely important to collect fifteen or twenty cc, or even a greater amount, of blood from the patient if possible, because usually there are only a very few organisms present in the blood stream and several tubes and plates must be inoculated because it is necessary to incubate the cultures under different atmospheric conditions. Enough blood should be available for guinea pig injection. The blood for cultures should be obtained in the early course of the disease at a time when the temperature of the patient is highest. We are of the opinion that the reason for so many failures to isolate *Br abortus* from the blood is that an attempt to recover this organism is not made until a search for every other infection has been completed. Anticoagulants may be used but there is a possibility that they may injure any organisms that are present. We have in several instances recovered *Br abortus* from samples of blood that were free from lithium oxalate and sodium citrate, while specimens that contained them developed no growth. We find as a rule that serum from coagulated blood is more satisfactory for serologic work than plasma from a sample to which some anticoagulant has been added.

THE AGGLUTINATION TEST

The agglutination test is no doubt the simplest and most satisfactory method of ascertaining whether or not a patient has undulant fever. One must keep in mind, however, that it has limitations and that frequently no agglutinins are present in the serum of patients suffering from the disease. Six per cent of the cases we have studied have fallen in this group. On the other hand, when the agglutinins have been formed, they usually remain in the blood a long time after the symptoms have subsided. We have tested the blood serum from three patients monthly for approximately two years and they still show comparatively high titers. One serum agglutinates the abortus antigen when diluted 1:45, while the other two show agglutination when diluted 1:405 and 1:1215. Quite frequently a serum is observed that shows a marked prezone, namely, the lowest dilutions of the serum which will not agglutinate the antigen, while perfect agglutination is observed in the serum diluted 1:400 or above this point. Therefore it is important to make a series of dilutions beyond this prezone to eliminate the possibility of reporting a positive serum negatively.

The technique of the agglutination test is identical with the usual technique employed for the Widal test. The use of dried blood, however, is quite unsatisfactory. We prefer to use the macroscopic tube test. In our serologic work we have employed an automatic pipetting syringe known as a rheometer in European laboratories, to avoid the possibility of laboratory infection.³ Our observations indicate that a living antigen standardized to give a reading of 3.5 cm. on the Gates apparatus is most satisfactory.⁴ We dilute the serum directly in the antigen which eliminates adding the salt solution to the tubes and diluting the serum in the salt solution. The tubes are then incubated for eight hours after which time readings are made. They are then set in an ice box for twenty-four hours when they are observed a second time for any delayed reaction. Because abortus agglutinins are usually reciprocal with those produced by *Br. melitensis* and occasionally with those produced by *Br. tularensis*, the serum should be set up also with antigens prepared from these two organisms. It is impossible to designate any specific serum titer for a positive diagnosis of undulant fever. Twelve of fifty serums from cases of undulant fever had a titer of 1:400 while the remainder showed titers above and below this dilution from no agglutinins to a titer of 1:32,805. We have observed severe cases of undulant fever with maximum titers of 1:15 and 1:30.

THE COMPLEMENT FIXATION TEST

The complement fixation test has no distinct advantage over the agglutination test. It is more complicated and often serums are found to be anti-complementary but still satisfactory for the agglutination test. The technique is identical with that of the standard Wassermann test except that an abortus antigen is used. King⁵ states that in many serums he has been able to get complement fixation before he could demonstrate agglutinins.

THE BLOOD CULTURE

Due to the variability of the atmospheric requirements of various strains of *Br. abortus*, cultures should be made by several different methods to iso-

late the organism successfully. Twenty c.c. of blood are collected from the patient and placed in a sterile tube, if it is not convenient to have the medium available for immediate use. As previously stated, we discourage the use of anticoagulants, although specimens of blood made outside of the laboratory may be cultured more easily if the blood has not coagulated. Coagulated samples must be broken up before satisfactory cultures can be made. Any meat infusion agar (preferably made from liver) with a P_H of 6.8 or 7.00 is satisfactory. Four tubes of slant agar may be inoculated and two plates may be poured, to each of which 2 c.c. of blood is added. The serum is collected from the remainder of the sample for serologic work and the clot or cells are injected into two guinea pigs, subcutaneously or intraperitoneally. Two of the slants and one of the plates are incubated in a jar in which 15 per cent of the air has been replaced by 10 per cent carbon dioxide. One of the slants is sealed with paraffin or sealing wax and placed in the incubator under normal atmospheric conditions with the unsealed slant and the other plate. If no growth is observed in seventy-two hours, the blood on the slant agar tubes should be smeared over the surface of the agar and reincubated. Cultures should not be discarded for twenty days, for growth of many of the strains of *Br. abortus* develops very slowly, and in one instance we failed to get a growth until after eighteen days. Recently we have been placing one or two c.c. of blood in 10 or 12 c.c. of liver bouillon, as suggested by Kristensen,⁶ who reports that he has been able to recover the organism in about 65 per cent of his cases. We have been more successful when we incubated the blood in the bouillon for seventy-two hours and then injected a guinea pig with 3 or 4 c.c. of the blood-bouillon mixture.

ANIMAL INOCULATION

We consider the guinea pig the most suitable laboratory animal for use in recovering pure cultures of *Br. abortus* from blood, tissues and milk, although Hagan⁷ has demonstrated that mice may be used and that the organism may be recovered from them in a much shorter interval of time than is required in the case of the former. The subcutaneous injection of body fluids and tissues is safer than the intraperitoneal method, but fresh blood from patients may be injected by the latter method without danger to the animal. Theoretically the guinea pig should become infected more quickly by this method, providing the blood has *Br. abortus* in it. The guinea pigs may be autopsied four or five weeks after their injection, although extensive lesions of the infection are not produced in such a short interval of time except in cases of very virulent strains. Usually two or three months are required for the infection to cause death and occasionally some guinea pigs recover from the disease if they are infected with cultures of low virulence.

After the guinea pig has died or has been sacrificed, the spleen is aseptically removed and placed in sterile Petri dishes for culture. A sample of blood for the agglutination and agglutinin absorption tests should be collected from the guinea pig's heart. A gross examination for abortus lesions in the liver, lymph nodes, reproductive organs, kidneys, and joints should be carefully made. The liver, lymph nodes, or abscesses may likewise be cultured but this is not necessary in a routine examination because the spleen is the

most suitable tissue from which to recover *Br abortus*. Bits of the spleen, the size of a pea, are planted on suitable media and incubated, as are the blood cultures, in jars containing 10 per cent carbon dioxide. If no growth is observed in three or four days after incubation it is well to smear the pieces of spleen over the surface of the media and reincubate the cultures. The identification of the culture recovered and of the antibodies in the serum is made as described in other sections of this report.

IDENTIFICATION OF CULTURES OF *BRUCELLA ABORTUS*

If bacterial colonies appear on the poured plates or a growth on the tubes they are picked and transferred to nutrient agar slants to which sterile horse serum has been added. They are then incubated at 37.5° C under the atmospheric condition which grew them best. After a suitable growth is obtained, the various 1 per cent carbohydrate media are inoculated. A broth culture is likewise inoculated to be examined for motility. Smears from colonies should be made and gram stained, and any *abortus* bacilli should appear as short gram negative rods or coccobacilli. It is to be remembered that the morphology of these two organisms is variable and that they have a pleomorphic tendency. The first few generations may show organisms that are almost spherical. On the other hand we have observed cultures, smears from which showed gram negative rods that seemed too long possibly to be *Br melitensis* or *Br abortus*. An antigen is prepared from the growth and an agglutination test is made with an anti-*abortus* serum. If the organisms are *Br abortus* or *Br melitensis*, it should not ferment any of the carbohydrates should be nonmotile and should be agglutinated by the antiserum. An agglutinin absorption test is then made to determine whether the organism recovered is *Br abortus* or *Br melitensis*. This is accomplished by incubating an anti-*abortus* and an anti-*melitensis* serum with large numbers of the unknown organism to absorb the antibodies in the serum specific for the organism.

THE AGGLUTININ ABSORPTION TEST

When the serum from a patient agglutinates both *Br abortus* and *Br melitensis* it is necessary to employ the agglutinin absorption test to determine which organism is responsible for the infection. The technique that we have used is as follows. Cultures of *Br abortus* (80) and *Br melitensis* (428) are grown on the surface of infusion agar in Blake bottles. After forty-eight or seventy-two hours' incubation at 37.5° C the growth is washed off with a small amount of a physiologic salt solution and centrifuged for one hour in graduated centrifuge tubes at 2000 R P M. The supernatant fluid is poured off and about 0.4 c c of the packed down organisms are allowed to remain in the tubes. To obtain this amount may require removal of part of the substrate or the addition of more organisms and a second centrifuging. One c c of a 1:5 diluted serum to be tested is placed in each of two centrifuge tubes, one containing 0.4 c c *Br abortus* organisms and the other 0.4 c c *Br melitensis* organisms. If the serum has a low titer, it should not be diluted. Four tenths of a c c of the organisms are a very satisfactory amount for a serum with a titer of from 1:400 to 1:1200, a serum with a higher titer should be diluted ten or twenty times. The serum and organisms are thoroughly mixed, incubated at 37.5° C for one hour and then refrigerated overnight. The next morning

the serum and organisms are again centrifuged for one-half hour at high speed. The absorbed serum is removed and set up with antigens prepared from the same cultures of *Br abortus* and *Br melitensis* as were employed for the absorption of the antibodies in the unknown serum. Agglutination of the abortus antigen caused by a serum absorbed by *Br melitensis* indicates that the agglutinins are specific for *Br abortus*. However, if agglutination of the melitensis antigen was caused by the serum absorbed by *Br abortus* the agglutinins are specific for *Br melitensis*. *Brucella melitensis* will remove some of the agglutinins from an antiabortus serum and *Br abortus* will remove some of the agglutinins from an antimelitensis serum, while each will remove all of the agglutinins from its specific antiserum. The method for identifying unknown cultures by agglutinin absorption is the same as above, except that one must have available known antiabortus and antimelitensis serums. Large amounts of the unknown culture are grown in Blake bottles, harvested and then incubated with the known antisera.

THE BLOOD COUNT

The blood count is of great assistance in establishing a diagnosis of undulant fever. In very mild cases the blood picture is not changed, but if the symptoms of the disease persist for several weeks, marked changes are observed which cannot be considered specific for undulant fever but furnish corroborative information that aids in making a diagnosis.

An extensive series of blood counts on twenty patients with undulant fever showed a secondary anemia and a leucopenia, with a relative and absolute lymphocytosis as constant changes. The percentage of hemoglobin often drops to 60 per cent and even to 50 per cent, as determined by Sahli's method in severe prolonged cases of the disease. The red cells in one case dropped from normal to 2,872,000 while the average low red cell count was approximately 3,600,000 per cmm. The lowest white cell count was 2,400 cell per cmm while the average was 4200 cells per cmm. The most marked change in the blood picture is seen in the differential count. The highest percentage of lymphocytes observed was 78 per cent with 22 per cent polymorphonuclear neutrophils. The average number of lymphocytes was approximately 45 per cent. Some reports have stated that there is a marked increase in the percentage of large monocytes. Our data do not show such evidence. In two cases 24 per cent and 25 per cent large monocytes were counted respectively. One case had been diagnosed as infectious mononucleosis. Of course there is a possibility of both diseases occurring at the same time in one individual. In the majority of cases studied the percentage of large monocytes has remained practically normal. With the increase in the number of lymphocytes, the polymorphonuclear neutrophils have decreased proportionally. In one instance of a fatal subacute endocarditis due to *Br abortus* the white cell count was 11,600 with 86 per cent neutrophils.

DISCUSSION AND SUMMARY

It is evident that many cases of undulant fever have been diagnosed as other infections in the past, but now the tendency is to base a diagnosis of this disease upon too little evidence. Unless the symptoms are very typical of undulant fever, agglutination of the abortus antigen by the patient's serum is

not a safe criterion from which to draw conclusions, because of the fact that in many cases the antibodies remain in the blood stream months and even years after the symptoms have subsided. However, the fact that certain patients show no antibodies is a limitation of the agglutination test that must not be forgotten. With our present knowledge of cultivating *Br abortus* from human blood, its isolation in every case seems impossible but a positive blood culture gives the most reliable information. There is no doubt that in the future a greater percentage of positive cultures will be isolated from specimens of blood from suspected cases of undulant fever. More laboratories are becoming familiar with suitable methods of growing *Br abortus* and *Br melitensis*. Until comparatively recently only a few laboratories engaged in the study of infectious abortion were familiar with the peculiar cultural characteristics of the organisms in this group. In our series of the first fifty cases studied we succeeded in obtaining cultures from only one third of the patients. We are now able to grow cultures of *Br abortus* from about 60 per cent of the samples submitted for bacteriologic examination. Kristensen reports that he has succeeded in recovering cultures from 65 per cent of his patients.

Because of the varied symptomatology of undulant fever and the limitations of the agglutination test, and because *Br abortus* is not isolated from cases of this disease it is very important that both the bacteriologist and the clinician have as much information concerning the patient as possible before a diagnosis is made.

Although great care should be exercised in handling cultures of *Br abortus* and *Br melitensis*, we believe that technicians and bacteriologists should not be unduly alarmed concerning the danger of working with these species. They cannot be so dangerous to work with as *Bacillus anthracis*, *Pfeifferella mallei* and the virus of rabies. As everyone knows, unnecessary fear causes more catastrophes than intelligent care in handling such infectious agents.

It appears that we have only made a beginning in the study of this disease. The bacteriologist must possess patience and perseverance, as well as a thorough knowledge of the cultural characteristics of *Br abortus* and of its serologic peculiarities to be of value to the practitioner in establishing a correct diagnosis of undulant fever.

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SOME OBSERVATIONS ON THE AGGLUTINATION OF BR ABORTUS*

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DURING a period of eighteen months, seven agglutinations for Br melitensis, var abortus, have been obtained in studies made in this laboratory out of 100 agglutination tests for that organism. Two were on patients admitted to the hospital, one a clear-cut medical case and the other admitted for tonsillectomy, following which, due to continued fever, this infection was discovered. The other agglutinations were done on sera submitted to the laboratory.

Both cases were considered as possible typhoid infections, but after negative findings the sera tested were agglutinated by Br abortus, the adult medical case in a dilution of 1:1280 and the child of fifteen years in a dilution of 1:640.

From this experience developed a desire to prepare a safe, fairly stable and readily agglutinable antigen to be used parallel with the Dreyer method for typhoid agglutination. Our first method has continued with practically no change, and it is possible that our experience may be profitable to others.

Three strains of Br abortus were obtained from the Laboratories of the State Live Stock Board, and a bacterial suspension of each was used against a known positive serum. The one giving the highest titer and clearest cut reaction was selected for stock culture. It is carried on 3 per cent glycerine, 1 per cent glucose agar.

The organism for antigen is incubated seventy-two hours on slants in large tubes (approximately 15 by 2 cm) on 3 per cent glycerine, 1 per cent glucose agar. Six to 8 slants will yield about 200 c.c. of antigen. The growth is then emulsified in 0.85 per cent NaCl and killed by heating in a water-bath at 55° C for forty-five minutes. It is then controlled for sterility and filtered through sterile cotton, shaken in a vaccine bottle with beads, and diluted with 0.85 per cent salt solution to an opacity equal to about 3 billion staphylococci per c.c. We store this antigen in a refrigerator, and have used no preservative, but 0.5 per cent phenol could probably be added without loss of antigenic value.

The test is set up in tubes 75 by 14 mm in size, providing four rows of 10 tubes each as for a Dreyer-Widal, the last row being for Br abortus. The patient's serum is set up in all rows in dilutions of 1:10, 1:20, 1:40, etc., and to the first row 0.75 c.c. of B typhosus antigen is added, to the second, Para A, to the third, Para B, and to the fourth Br abortus antigen. Place in a water-bath at between 50°-55° C (best about 52° C) for two hours.

As a check on this test, 10 control tubes are included, using a negative serum plus saline, a positive serum plus saline, a positive or immune serum,

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saline and respective antigen for each row and the same with a negative serum. After two hours remove rack from the water bath, take preliminary reading and place in the ice box for six to eight hours, or better, overnight. We have found this has given clearer cut reactions or has sometimes raised the titer one or even two dilutions.

The reactions are read on the usually accepted basis of 4+ for complete flocculation and clearing, 3+ for 75 per cent clearing, 2+ for 50 per cent clearing, 1+ for partial clearing and 0 for no reaction.

Although our experience has been limited in the discovery of so few positive infections we have considered much of the literature on this infection and have used other recommended methods for this agglutination. Our method seems to have given reliable results on a minimum of added procedure with no special equipment, no separate test, no seemingly difficult standardization of an antigen.

In comparison with other methods we have made the following observations on all sera submitted during eighteen months on which Widal and Br abortus tests have been performed.

1 Antigen, prepared as indicated has given clear cut reactions at least two months after its preparation, and tests done on ice box stored antigen over five months old have given 90 per cent clearing on the same sera. Titer on two months old antigen was unchanged. Handled under sterile precautions no contamination has occurred.

2 In a study of system controls the following observations have been made on positive sera.

a In all typhoid and paratyphoid agglutinations the reaction was definite or almost complete in the first fifteen minutes of incubation.

b In Br abortus strain, agglutination did not begin earlier than one hour after incubation had begun, and continued slowly during the next hour. It was complete in two hours.

3 Relative character of agglutination.

a In all typhoid strains the bacterial agglutination was light feathery, and on agitation returned to almost complete bacterial suspension.

b With Br abortus the bacterial agglutination was coarsely granular, and on agitation did not return to suspension. It settled again almost immediately. We think this an important and useful observation.

4 Time of incubation. It is believed that this period is sufficient for agglutinations within the range of any dilution accepted as of clinical significance. We have never obtained an agglutination in a dilution of less than 1:20. We have reported none on a dilution less than 1:50 and then with reservation.

5 Inactivated sera. We have not inactivated our sera on routine tests. Studies were made on 25 sera, both inactivated and uninactivated, and this method has shown no difference in results. All were negative down to 1:20 dilution.

Reference to the literature shows a variation in the recorded thermal death point of Br abortus from fifteen minutes at 60° C. to two hours at

60° C These results in many cases refer to suspensions in milk We have found that our heavy suspensions in salt solution have invariably been killed by heating for forty-five minutes at 55° C, and feel there is probably less alteration of the bacterial structure at that point than at higher temperatures

The incidence of human infection with *B1 abortus* is variously indicated in different reports This is probably not due entirely to local variations in the prevalence of the disease, but partly to the various groups from which the material is drawn

McAlpine and Wedeman¹ report a 0.6 per cent incidence of human infection with *B1 abortus* in a state in which 90 per cent of the cattle were found to be infected and only 60 per cent of the milk pasteurized This figure represents the positives obtained in a study of over 10,000 human sera submitted for Wassermann test

Our series, on the other hand, represents about 7 per cent positive agglutinations for *Br abortus*, in a series of 100 cases, most of whom had febrile symptoms, and many of whom were at first thought to be typhoid fever It is obvious that the incidence would be higher in such a group

In the inactivation of sera, Négie and Ravnaud² cite instances of false positives on unheated sera, which are avoided if sera be heated to 56° C Evans³ reports in her study that inactivation did not reduce percentage of positives and Hardy recommends inactivation at 56° C for thirty minutes, if a 37° C water-bath be used for the test

We might remark here that although we have not inactivated our sera, we feel that 50 to 55° C for two hours has inactivated our sera within the first twenty minutes If bacteriolysis occur during this time it is probably slight

We have used in our positive control study an immune serum now a year old This serum certainly contains no complement

Rapid macroscopic agglutination The method of Huddleson⁴ has been studied on a limited number of cases It has not discovered any positives other than those we found by our method, which in addition gives a titer on the case This method is doubtless useful where many sera are to be examined The higher percentage salt solution (12 per cent) renders the antigen likely to crystallization, a disadvantage where occasional tests are made We did not get clear-cut reactions except in cases of positives of high value

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NOTES ON THE BACTERIOLOGY OF THE BRUCELLA GROUP*

By K. F. MEYER AND B. EDDIE SAN FRANCISCO CALIF

IN THE study of infectious diseases it is the aim of the pathologist to recognize the significance of finer distinction between strains of pathogenic organisms, the purpose and the limitations of the response to infection by the host, and the process of stimulation and adjustment of immunity reaction to meet the peculiar conditions of the case. These fundamental prerequisites must be applied to the study of the problem of undulant fever and the organisms which cause this disease. Only too frequently the early inquiries are inspired by the needs of diagnosis and the wish to prevent or to cure, and relatively little is done in the analysis and the interpretation of the process and progress of the infection. When the authors resumed, a few years ago the study of undulant fever, it was realized that much could be gained if the inquiries would be disinterested and would primarily deal with an investigation of the tolerance and antagonism that the specific organism meets in animals which it infects. Although primarily interested in this phase it became necessary to compare the cultures which were used in the study. Since previous papers from the Hooper Foundation had shown that the main representatives of the Brucella group, *B. abortus* and *B. melitensis* cannot be distinguished by morphologic or biochemical criteria, the agglutinin absorption test was chosen as the method of differentiation. First employed by Feusier and Meyer¹ and then developed by A. Evans on a broad basis this procedure led to the recognition of at least eight serologic groups. Every strain used in the experiments on animals has been compared with at least two authentic strains representing the main varieties observed in the United States. Contrary to previous experiences the final identification was very difficult since a number of new serologic nuances and varieties could not be correlated with the standard types. Particularly the strains from European countries have shown many intermediate types which still await final serologic identification. Some are new *melitensis* types others are probably subvarieties of the *para abortus* group. Until the time consuming studies have been completed, it is impossible to decide the pertinent question. Is the serologic classification a superficial and perhaps a valueless complication which does not coincide with the invasive power and the pathogenic behavior? The author is prepared to admit that he has overestimated the significance of the agglutinin absorption test. It is not unlikely that the Brucella group of organisms is derived from a common stock which has differentiated and is still differentiating in their relationship to man and animals. In the environment in which the author has made most of his investigation the *abortus*

*From the George Williams Hooper Foundation for Medical Research University of California

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variety is responsible for the placental disease of cattle, but Evans,³ Huddleson,⁴ and others have proved directly or indirectly the occurrence of true melitensis varieties in bovines. Therefore, the general assumption that the true melitensis is primarily a parasite of the goat and the patients with melitensis septicemias have been infected through caprine sources may need revision. Thus it must further be concluded that the serologic identification, if successful with the authentic strains at hand, does not in all probability determine the source of the variety. In other words, the isolation of a melitensis from the blood of a patient is no proof that he or she contracted the disease through goat's milk or goat's milk products. Furthermore, the demonstration of a melitensis variety in cow's milk does not establish the goat as the source of the bovine infection. In fact, it is as yet unknown where and how these bovine-melitensis strains first originated. It would be interesting to study the serologic behavior of a B₁ melitensis after prolonged sojourn in an aberrant host like the cow. Burnet⁵ has investigated the effect of the abortus on goats, and although he found the organism to be less adapted to this ruminant, he has not considered the possibility of an adaptation through passage.

The abortus varieties have remained cosmopolitan in their activities, they infest aside from cattle, hogs, sheep, and horses, and in place of acquiring a more specific virulence, they have widened the range of their less specialized attack, and developed a more potent toxin. This is indicated by the pathogenic behavior of the abortus strains of porcine origin which has been carefully studied by Th. Smith⁶ and amply confirmed by other workers. The selection of the guinea pig to differentiate the porcine from the bovine strains has added a new method to the procedures which may be used in the classification of the *Brucella* organism of human origin. These pathogenicity tests established B₁ abortus of porcine origin as the causative organism of human undulant fever. But since a number of workers have found this variety which cannot be separated from the true bovine abortus by agglutinin absorption tests in cow's milk, the old question, "where did the human patient contract his infection?" remains undecided. Furthermore, the pathogenicity of the abortus strains of porcine origin may be low and resemble that of the bovine varieties (Smith⁷). Under these circumstances, differentiation by animal tests may be difficult. In this connection, it is recalled that the pathogenicity of a human *Brucella* organism recently isolated from a case of undulant fever in the southwestern part of the United States may be feeble or nil for guinea pigs or a strain, serologically a true melitensis, may a few days after its isolation produce typical bovine lesions. These observations, kindly confirmed by Dr. Th. Smith impose certain limitations on the pathogenicity tests for differential purposes. Equally unsatisfactory have been the thermoagglutination tests as advocated by Fican and Alessandrini,⁸ the differential allergic reactions (Fleischner and Meyer,⁹ Poletti,¹⁰), the cross immunization experiments of Zibordi,¹¹ the nonspecific acid agglutination test with different H-ion concentrations of lactic acid according to Verzellana and Zanzucchi¹² (see also Beguet,¹³ La Rosa,¹⁴ Ceruti,¹⁵ Favilli¹⁶ and Andrei¹⁷), and the thermoprecipitation reaction of Valenti¹⁸. Personal experiences with the

thermoagglutination and the Valenti test justify the conclusion that they are both valueless

At this stage of the investigations, studies on the nutritive substances, growth accessory factors required by the *Brucella* organisms, the effect of salts, the nature of the enzymes, etc., have been undertaken. The work will be reported when completed. To date it has not furnished any definite proof that biologic means may be found which may readily distinguish the varieties of the *Brucella* genus. The experiments supply, however, important information regarding the virulence and toxin production in relation to the culture medium. While working on these phases of the problem two new methods have been proposed for the differentiation of the varieties of the genus *Brucella*. One developed by McAlpine and Slanetz¹⁹ makes use of the differences in the glucose and nitrogen metabolism which can be tested in broth cultures of the *Brucella* organism and the other, recommended by Huddleson,²⁰ determines the growth differences of the species by use of dye media. According to the first named workers, certain striking differences in the utilization of glucose by the varieties of the *Brucella* group show that the abortus melitensis group may be subdivided into two main divisions. The first, known as the abortus group, comprises strains which are unable to utilize more than 2 per cent glucose in the presence of Fairchild's peptone and consequently, demerize the amino acids and render the medium increasingly alkaline. The second group includes the porcine and melitensis strains which hydrolyze from 5 to 20 per cent of the carbohydrate and during the first six days of growth increasing acid values over the control figures may be recorded.

Later, McAlpine and Slanetz²¹ found that these differences in the metabolism are closely paralleled by the behavior of the strains toward carbon dioxide. Thus the growth of the abortus cultures is accelerated by the cultivation in an environment containing 5 to 10 per cent CO₂ while the porcine and melitensis cultures are more or less inhibited when incubated under the same conditions. In brief, distinct metabolic differences exist between the porcine and bovine varieties. However these procedures fail to differentiate between the porcine and melitensis varieties. Huddleson²² has supplemented these shortcomings first by a study of the rate of H₂S production and then by a comparison of the genesistatic properties of certain dyes to interfere with the reproductive mechanism of the representatives of the genus *Brucella*. According to the time and rate of H₂S production genesistasis toward thionin, methyl violet and basic fuchsin he subdivides the *Brucella* into three species: Br abortus, Br suis, and Br melitensis. Whether or not this classification is justified remains to be determined. The designation, Br suis, is probably premature since recent observations indicate that swine may become infected with the true abortus indistinguishable from the bovine variety. Tentatively the author has adopted the terminology of Huddleson to record concisely the selective genesistatic potency of certain dyes on the representatives of the *Brucella* group: Bovis thionin genesistatic fuchsin and methyl violet fast Suis, thionin fast but fuchsin and methyl violet genesistatic Melitensis thionin, fuchsin and as a rule, methyl violet fast. But since the mechanism of the selective bacteriostasis is unknown, it is believed that this interesting method

should not be used indiscriminately in the determination of the possible source of undulant fever in man

During the past year the procedures of McAlpine and Slanetz and those of Huddleson and his associates have been used in the Hooper Laboratories and are the subject of biochemical analysis. The glucose utilization tests have been supplemented by a determination of the nature of the fixed and volatile acids which are formed by the various *Brucellas*. The data have been collected by a worker who was not acquainted with the histories or origin of the cultures. Recently some of his findings have been correlated with the serologic data and the results on the dye plates. The duplication of the differential test devised by Huddleson at first offered considerable difficulties since Giubler pre-war and Coleman and Bell dyes were used. After being advised by Dr. I. F. Huddleson that he works entirely with Certified National aniline dyes, the behavior of certain authentic strains was found to correspond more closely to those published by him. Since the observations with different dyes will be the subject of a separate publication from this laboratory, it is unnecessary to enter into a discussion of the discrepancies. Suffice it to indicate that for example the Giubler thionin was rarely inhibitive for the "abortus" while the Coleman and Bell dye even exerted a bacteriostatic effect on the "suis" strains. In all probability the presence or absence of impurities may control the selective genesisstatic effect of certain dyes. It is the purpose of the biochemical studies which are now in progress to determine the nature of these substances. Until the value of this differential method for the *Brucella* group has been determined by workers throughout the world, it is important to specify the use of National aniline and not merely certified dyes. If this prerequisite is not considered results as those recently published by Saitta²² from the laboratory of Professor Poletti in Cagliari in Italy will rapidly discredit a test which deserves further study.

Over 130 cultures of *Brucella* have been tested at least three and some six times on media containing different dilutions of the dyes. Certain strains have shown variable sensitiveness toward thionin. Recently isolated organisms classified serologically as *melitensis* may be inhibited by gentian-violet in a dilution of 1:50,000 and 1:100,000 and give faint growth in 1:250,000. As a rule, the behavior toward basic fuchsin has been rather constant. The strains either grow in every test or they are regularly inhibited. However, reduction of the dye may accompany the growth of certain abortus and few *melitensis* strains. The significance of this phenomenon is unknown.

Some of the observations worthy of note are briefly as follows. Twenty cultures from bovine and one from porcine sources in Switzerland may be classed as "bovis". Of eight cultures obtained from Germany as true "abortus" strains, three behave like "melitensis" and five like bovis types, of four Hungarian cultures, one behaves like a *melitensis* while three Italian cultures are identified as "bovis" varieties. Equally interesting are the findings on the human cultures. In general, the strains classified serologically as "melitensis" reacted as such on the dye media, those identified by agglutinin absorption tests as abortus were either linked with the "suis" or the "bovis" types. With one exception, twenty cultures sent by Dr. A. V. Hardy from

Iowa are "suis" varieties, some of the New York strains fall into the same group while the Michigan cultures are mostly "bovis" types. Twelve strains isolated by Dr M. Kristensen in Denmark and one human strain obtained from Dr H. Habs and isolated at Kiel, Germany, react like "bovis" and four recent cultures from Tunis like "melitensis" types. However, one recently isolated California strain and an old laboratory culture (426, out No 20 Austria) serologically abortus varieties, reacted like "melitensis". According to Huddleson, an equine strain studied by A. Evans as a melitensis possesses also a dye sensitiveness like a "bovis" type. Through the courtesy of Dr Huddleson, the behavior of the irregular German, Hungarian, Austrian, and Californian strains toward dyes has been confirmed. Strain 6454 from a bovine placenta utilized 3 to 5.4 per cent glucose in seven days, is inhibited by 20 per cent CO and is serologically an abortus. The two other German and one Hungarian abortus obtained from fetuses gave repeatedly low glucose utilization figures and grew well at 20 per cent CO. The California strain (Love) hydrolyzes 4.2 per cent glucose, grows freely in CO and produces a progressive amount of H₂S in liver and in cystine veal infusion agar. It produces "bovine" lesions in guinea pigs but infects monkeys like a "melitensis".

As a whole, the study of the glucose metabolism has not furnished the differences one would expect from the publications of McAlpine and Slanetz. Quite a number of abortus cultures utilize more than 2 per cent while several "melitensis" strains split the carbohydrate in amounts below 5 per cent. On the other hand it is evident that the feeble biochemical activity of the majority of the abortus strains may be contrasted by the vigorous carbohydrate utilization of certain porcine and the Tunisian melitensis cultures. The two main groups are, however, joined by strains which from a differential diagnostic standpoint consume an indecisive amount of glucose. It may be mere coincidence but the biochemically aberrant strains reveal also a sensitiveness to dyes which cannot be correlated with the serologic reactions. What is the meaning of these biologic nuances? Are they new species in the course of formation or are they new host adaptations? The limited data hardly justify conclusions. However it is evident that the previously expressed view concerning the plasticity and variability within the Brucella group is again expressed in the biochemical and dye sensitiveness tests. Until their origin is known, their importance in the epidemiology of undulant fever belongs to the realm of speculation. Even if it is assumed that Brucella organisms which according to the differential tests belong in the "melitensis" group may infect cattle and consequently man it still remains to be determined why for example in the observation of Carpenter and King²³ only 6 of 150 or more persons who partook of the milk became infected or why in the case of Frei²⁴ the milk of an abortus infected goat was ingested with impunity by a volunteer. In all probability the problem is not so much the discovery and classification of the varieties but the recognition of the tolerance and antagonism that the Brucella meets in the animals or human beings it infects. The low human population susceptibility which according to Orr and Huddleson⁵ is only 1.4 per cent, when exposed constantly to the abortus organisms has, as far as is known, not received further investigation. If only those individuals

who develop agglutinins or clinical manifestations of the disease are to be considered infected, then it must be held that the vast majority of persons in northern countries in which the placental disease of cattle is common, possess a tremendous natural immunity against the Br abortus and its varieties. From a clinical standpoint, this view may appear justifiable but bacteriologically other possibilities exist. Either the constant ingestion of a small number of Brucella organisms in cow's milk during infancy may lead to an acquired immunity or a carrier state. In this connection the discovery of the abortus bacillus in the tonsils of children by Mohler and Traum²⁶ in 1911 deserves further investigation. From this point of view probably few people exhibit any natural immunity and whether they develop signs and symptoms of the disease depends upon the intensity of their exposure to the infection and the reaction of the host. The nature of the main defense and the mechanism leading to the immunity are not known, although Ninni²⁷ in Rome has recently demonstrated a strong bactericidal action of normal human sera on the Bang's but not on the majority of the Bruce melitensis bacteria. It is these phases of the undulant fever problem which have interested the workers in the Hooper Foundation. Preliminary to the studies on man the susceptibility of monkeys to the representatives of the Brucella group has been investigated on 88 monkeys. The following observations have been made.

A single oral administration of 21 different Br abortus strains produced in 24 Macacus rhesus and 1 M. cynomolgus monkey nonfebrile infections, followed by the formation of specific agglutinins of moderately high value. The dosage varied from 7 to 400 millions and in some experiments it consisted of many billions. The course of the clinical disease is not markedly influenced by the number of bacteria which are fed. Two cynomolgus and one rhesus monkeys were refractory. The strains identified serologically as abortus or para-abortus varieties and in the dye test as "bovis" or "melitensis" types had been isolated from bovine pathologic specimens in the United States, Germany, Hungary, Italy, and Switzerland. Blood cultures have not been successful. The value of the serum agglutinins and their persistence depends on the feeding dose. Rapid disappearance of the agglutinative power to a low titer or to the zero point is worthy of note. A cutaneous application of approximately 20,000 bacteria has induced an infection. The incubation period as indicated by the appearance of the serum reaction varied from nine to thirty days and is influenced by the infective dose. In general, the smaller the dose the longer the incubation time. The absolute evidence of infection has been secured through the recovery of the organisms from the tissues of four monkeys which have been sacrificed on the thirty-fourth to fifty-second day. Three animals killed on the forty-third, fifty-sixth and one hundred ninety-ninth day furnished sterile cultures. Probably every Br abortus strain when fed in sufficiently large dosage is pathogenic provided susceptible monkeys are used. It is conclusively proved that even old laboratory strains may penetrate the intestinal mucosa and stimulate the production of antibodies. In general, the validity of previous conclusions that the Br abortus is less pathogenic than Br melitensis is confirmed, but the differences are less marked.

probably on account of the greater variety of cultures, and the larger series of animals which has been studied

By feeding 100 million *Br abortus* type "sus" of bovine but in all probability of porcine origin which has retained its characteristics through the passage, a febrile disease with anatomic lesions indistinguishable from those of a *Br melitensis* infection of the monkey has been produced. Repeated feedings of 35,000 bacteria, cutaneous application to the shaved skin and intravenous injection of the organism has given the same result. Although the blood stream sterilized itself rapidly, the causative *Brucella* organism is readily isolated in enormous numbers from the hemopoietic organs and lymph nodes provided the monkeys are sacrificed within thirty to fifty days after the administration of the infective material. The incubation time dependent on the dose varied from six to twenty one days. The maximum value of the agglutinative power of the serum in part controlled by the feeding dose persists for several weeks, and a somewhat lower titer for many months. However, the persistence is not any longer than that observed in monkeys infected with *Br abortus* or *Br melitensis*. During artificial cultivation the febrile properties on feeding have been lost but they have been retained by one strain when the organisms are applied cutaneously. The milk of the cow which furnished one of the pathogenic sus strains has been consumed by a group of people without any bad effects.

An old laboratory culture of a *Br abortus* type "sus" of porcine origin infected via the alimentary tract when fed in large doses. The infection ran an afebrile course, stimulated after an incubation time of from nine to ten days, a powerful agglutinative value of the serum with an abundance of specific organisms in the tissues.

A *Br abortus* type "bovis" isolated from a swine fetus infected and immunized a monkey in a manner similar to that of the 'bovis' types of bovine origin.

Melitensis strains of American origin possess a low virulence for monkeys, they may act like "bovis" cultures, and they may lose their pathogenicity entirely within six months of artificial cultivation. Test tube strains several years old are nonpathogenic and when administered by mouth they lack immunizing properties. One culture which produced no lesions in guinea pigs by injection, infected a rhesus by mouth.

Tunisian strains of *Br melitensis* fed or inoculated in doses of 100 million bacteria give rise to a febrile disease which is generally considered characteristic for this group of organisms. Even recently isolated strains may induce merely serologic but no febrile reactions.

A *Brucella* organism serologically and biochemically an *abortus* and in its behavior toward dyes a *melitensis* type acted like a typical *melitensis* by feeding and inoculation one month after isolation from a California patient with undulant fever. In contrast, nine other strains kept under artificial cultivation for from one to twenty four months and isolated from human *abortus* fever cases in Michigan, Iowa, Northern Germany and Denmark infect monkeys when fed in a manner characteristic for the *Br abortus* "bovis".

type Febrile reactions may be recorded on intravenous inoculation of large doses (one rhesus) No specific thermic response has been noted following the separate subcutaneous injection of two Danish human abortus strains

The agglutinative power of the serum with few exceptions remains low and is frequently of short duration even following the administration of massive doses via the alimentary tract

An old type culture No 20 (426, A Evans) originally from Austria, isolated prior to 1915, serologically an abortus and on dye media a melitensis type, readily penetrated the intestinal mucosa and immunized against a virulent infection with a "suis" type Three Br abortus type suis strains of human origin have not exhibited any striking pathogenicity or marked febrile properties, neither by feeding nor by cutaneous or intravenous injection They behave like test tube strains of the melitensis variety and show evidence of rapid deterioration under artificial cultivation A fourth culture with a history which suggests a laboratory infection with virulent *Brucella* organisms of porcine origin produced a typical *Brucella* septicemia with a marked pyrexia and a rapid serologic reaction following the oral introduction of 182 million bacteria

Serum agglutinins specific for the *Brucella* group are formed only in the presence of a definite infection The ingestion of heat-killed abortus bacilli with or without bile is antigenically ineffective in monkeys and rabbits

Over 10 per cent of the rhesus and cynomolgus monkeys possess a natural immunity against *Brucella* infections via the alimentary tract Animals which react to the oral administration of virulent abortus organisms with moderate and in general transitory serum reactions resist subsequent feeding infections with B1 abortus "bovis" and "suis" but not with a Tunisian Br melitensis The inherent and the acquired local intestinal immunity may be broken by an intravenous or subcutaneous injection of a virulent type "suis" or "melitensis" strain An alimentary introduction of living and moderately virulent *Brucella* organisms and then varieties may readily vaccinate rhesus and cynomolgus monkeys The protection thus afforded is relative and its mechanism deserves further investigation since it may explain certain puzzling features of the epidemiology of abortus undulant fever in the countries in which abortion disease in cattle is prevalent There are indications that previous contact with noninfective doses of *Brucella* organisms may render the animal hyperergic to massive parenteral infections Continuous ingestion of small numbers of abortus may lead to mild, unrecognized or "silent" yet immunizing infections At least in one observation, the local and general immunity thus induced has been definite

Final conclusions concerning the relationship of the different *Brucella* varieties to human undulant fever in this country cannot now be drawn The evidence incriminates B1 melitensis var abortus of bovine origin as the causative factor in a fairly large percentage of carefully studied cases But why and how these cases occur remains to be determined The bacteriologist who must assist the epidemiologist should appreciate that the cultures should be tested by the differential methods as soon as possible after their isolation In particular pathogenicity tests on monkeys and guinea pigs should not be

delayed. The behavior of these strains following bovine, caprine and porcine passage should be scrutinized and the process of stimulation and adjustment of the immunity reactions which the animal body may mobilize in order to ward off *Brucella* infection deserves detailed consideration. The pathologist's contribution to the problem of undulant fever lies in the careful analysis and interpretation of the process and progress of the infection in combination with the biologic analysis of the causative organism.

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DISCUSSION OF PAPERS BY GIORDANO AND SENSENICH, CARPLINTIR AND BOAK, LYNCH AND CALLAN, AND MEYER AND EDDIE

Dr Walter M Simpson—I was surprised to find my name on the program because I have no formal paper to present I have prepared a paper on undulant fever for the Section on Pathology and Physiology of the American Medical Association, to be read next week However, to open the discussion I will tell you some of our experiences with undulant fever During the past year we have carried out a clinical and pathologic investigation of sixty three cases of undulant fever in Dayton The experience of Evans, Carpenter, Huddleson, Hardy and ourselves would indicate that if an individual undertakes a survey of undulant fever in any given place, he will be surprised at the large number of cases which will be discovered It is generally known that infectious abortion of cattle and domestic animals is widely prevalent Our own experience would indicate that the disease is very common in southern Ohio Of sixty three cows in four herds that supplied milk to ten of the Dayton patients, all but four calves gave serologic evidence of Brucella abortus infection Five of these cows were eliminating the organisms in their milk in large numbers Cultures derived from the blood of one human case and from the milk of three of the cows were submitted to Huddleson, who found that they were all of the bovine type

As to the clinical manifestations of the disease they have been completely described by Dr Giordano It has been repeatedly stated that there is no well defined clinical picture of this disease Evans has stated that there is no other disease in which the clinician is so dependent on the laboratorian Our experience has not been in accord with this widely accepted belief An initial clinical diagnosis of undulant fever has been made by several Dayton physicians and they have asked us only to confirm their clinical diagnoses As regards the sexual incidence, we found both sexes to be about equally affected In six instances the disease occurred during the first decade of life, the youngest was a child of six The greatest incidence occurs between 20 and 40 Twenty two of our patients were housewives, only 9 were farmers or dairymen, seven were university students, and the remainder were engaged in nonagricultural pursuits More than one member of six families was affected Seven of the patients had been admitted to the hospital with a diagnosis of acute rheumatic fever, all showed acute joint manifestations Abdominal pain was a prominent feature of the disease in eleven patients, in five of these there was lower right quadrant pain with fever, appendectomy was performed in three cases In one of these an acute gangrenous appendix was found The other two appendices were entirely normal and cultures were negative In one case of upper right quadrant pain, accompanied by chills, fever and sweats, cholecystectomy was contemplated

In view of the fact that Brucella abortus derives its name from its known tendency to cause abortion in animals, we wondered whether or not it might be a factor in certain cases of human abortion We found five women who had repeatedly aborted, whose serum agglutinated Brucella abortus in titers from 1 80 to 1 320, four of the five gave a history of a previous febrile illness, the exact nature of which was not established at the time All were raw milk consumers

It is believed by many that the disease may be transmitted to cows by the bull through the seminal route Three of our male patients went first to genitourinary surgeons because of orchitis, epididymitis and prostatitis There was no history of gonococcal infec

ion and repeated examinations failed to reveal any gram negative diplococci. The sera of these men agglutinated *Brucella abortus* in titers of 1:160 to 1:640. In one of the cases we recovered *Brucella abortus* from a sinus tract extending through the scrotal wall.

In three of our patients there was an enlarged spleen and an eruption simulating the roseola of typhoid fever. Six of our patients were confined in a local tuberculosis sanatorium, all had been admitted with a diagnosis of tuberculosis.

There are a few cases in which anti-*Brucella* agglutinins do not develop. There appears to be need of a more delicate test to supplement the agglutination reaction. The intradermal test seems to hold considerable promise. In ten cases, with positive agglutination in titers from 1:160 to 1:1280 we carried out skin tests using 0.1 cc of a suspension of heat-killed organisms adjusted to the turbidity standard used in the preparation of antigen for the agglutination test. In each instance a strongly positive local reaction occurred. On the same patients, we later carried out a skin test with antigen sent to us by Giordano with similar results.

For practical purposes we adopted the standard of regarding all cases in which agglutinations occurred in titers of 1:80 or above, or in which we recovered the organism, as evidence of past or present infection with *Brucella abortus*. If an individual is sick and his serum agglutinates *Brucella abortus* it does not necessarily mean that that individual is suffering from undulant fever at the time of the test; the agglutination may be the result of a previous experience with undulant fever, months or years previously. In those cases in which the agglutination titer is 1:10, 1:20 or 1:40 further bacteriological and serological studies should be carried out.

As to the treatment, we have used in 29 cases a vaccine which was prepared from killed *Brucella abortus* organisms, standardized to 2 billion per cc. Graded doses were given beginning with one-fourth cc. at two or three day intervals. Following the first injections there was usually a transient rise in temperature. With three exceptions the fever rapidly returned to the normal level after two to six injections. Injections of milk or typhoid vaccine did not give equally good results. I am no vaccine enthusiast but I do believe that this matter is worthy of further study by other workers. It is a very difficult thing to estimate the value of any vaccine therapy in a disease which is characterized by spontaneous remissions.

Dr. Frank W. Hartman—I would like to ask if any of the essayists have seen this in children. We had a case in Detroit a year ago in a child eight years old. The parents insisted it had never had anything but certified milk. They made things very unpleasant for the dairies in the city of Detroit. That child responded to multiple transfusions.

Dr. C. W. Maynard—I simply want to ask a question and hope to be answered from the standpoint of the public health laboratory. We have about half of our milk supply pasteurized and the other half from raw milk dairies. For two years we have looked for undulant fever, not in as concentrated a way as some of the men have been doing, but we have been looking for it and have been using antigen from the hygienic laboratory. We had one case that has been proved. Should we public health officers insist upon the other half of our milk being pasteurized? Many prefer raw milk. When we have these cases how long should we keep them under public health supervision? Should we be lenient with them or keep them under supervision as long as we can isolate the organism from the urine?

Dr. R. L. Sensenich—It has been well pointed out in this symposium that the clinical course of the disease may make the diagnosis possible or the absence of characteristic evidence may throw the diagnosis into the realm of the clinical pathologist. It is our experience as Dr. Simpson has reported in his series of cases that the diagnosis in a great many instances may be made clinically provided an educational campaign is carried on among the physicians of the community as I understand Dr. Simpson has done. The illness may be very short and the character of the infection unsuspected. Dr. Lynch's presentation is interesting as I believe that some of these cases of short clinical course ultimately appear among those in whom the blood findings may be positive and yet there may be no other evidence of undulant fever. The cases of more protracted illness usually display clinical phenomena which are sufficiently characteristic to make the diagnosis comparable to that of typhoid fever in which clinical conclusions are confirmed by laboratory tests. In

the acute cases the situation is different. However, any acute septic type of illness with chills and fever, without readily demonstrable infection, should suggest undulant fever and indicate the advisability of blood studies. The presentation of the paper of Dr Giordano's and mine was an effort to further describe and classify the clinical types of the disease encountered and point out the frequency of a relatively short, acute course in the belief that the diagnosis will be more often made if physicians have the possibility of undulant fever in mind. Specific treatment has been unsatisfactory. Nothing has taken the place of bed rest. Fortunately, a surprisingly large number of cases run a very limited course and make a complete recovery.

Dr A V Hardy—In our study of undulant fever in Iowa we have been impressed with the fact that time after time we would come to farms and find the man infected and the woman not involved. The proportion of infected men to women living on farms is 9 to 1. Other investigators in the United States have had similar findings. This observation led us to believe that the skin might be the portal of entry. We undertook an experimental investigation of this point. We used guinea pigs and we selected two organisms, one a porcine strain which gave very marked pathologic lesions, and the other the bovine type. We prepared the organisms by growing on agar for forty-eight hours. We washed these off and diluted to compare with the 500 ppm opacity standard. We used four different methods of exposure. First, shaving the skin and abrading. Second, carefully shaving the skin, no apparent abrasion. Third, clipping the hair. Organisms (0.2 cc) were applied and spread over the prepared area with a glass rod. The fourth group we fed the same amount by mouth. We were quite surprised with the results. Of the pigs in which the skin was shaved and abraded, 100 per cent of the pigs were infected, those shaved with no abrasion, 90 per cent were infected, those with the hair clipped, 78 per cent, but in those fed by mouth only 22 per cent. Four times the number were infected by contact with the skin than with feeding. Dosage has a great deal to do in determining whether infection will be required and further experiments confirmed this belief.

We were particularly fortunate also in being allowed to study a group of packing house employees. We had diagnosed seven cases in that packing house and the men in charge agreed to ask the men to report for blood examinations. We found of the 217 bloods obtained, 29 or 14 per cent reacted in the titer of 1:80 or higher. The proportion infected varied depending on the intimacy of contact with fresh tissues. Less than half of those giving laboratory evidence of infection gave any history of undulant fever and only three had had clinical diagnoses.

I feel that the portal of entry must not be considered a closed question. It seems clear that there is more than one portal of entry, and we should study to determine the relative importance of the different sites of entrance of infecting organisms. The skin as a portal of entry must be given more consideration.

Dr C W Bonyng—Regarding joint involvement in undulant fever—I think we have had twelve to fifteen cases of undulant fever in Los Angeles, and in speaking with Dr Hammack, we both remarked that none of these have shown joint involvement. I am glad this point has been brought out by the speaker.

Dr Maynard has also brought out an interesting point, the occurrence of undulant fever in infants. We have been led to believe that infants are immune. We had one case in an eleven months old child who had been on certified milk for nine months. It would be hard to question that this was a milk borne infection. However three other children in the same family, using larger quantities of the same milk, were not infected.

It would seem that we still have much to learn regarding undulant fever, especially of the etiology.

Recently the Los Angeles Certified Milk Commission has ruled that our certified dairies must rid themselves of infected animals as determined by the agglutination test. The herds are repeatedly retested and only negative animals may be added. Since May, 1929, no certified milk has been served except from negative cows.

The certified dairies of the San Francisco district have been abortus free for nearly fourteen months and continuous examination of the milk has given negative results for *Brucella abortus*.

Dr Charles M Carpenter—I have isolated *Brucella abortus* from seven of fifty five pairs of tonsils. We have found the tonsils and lymph nodes of calves to become infected first and to remain infected the longest of any of their tissues. I want to mention a marked difference in the results obtained by Huddleson from those reported by McAlpine and Slanetz. The former stated that according to the inhibitory effect of dyes on *Brucella melitensis* it is similar to *Brucella abortus* of bovine origin while the latter, who studied the utilization of glucose by *Brucella melitensis*, *Brucella abortus* of bovine and porcine origins reported that *Brucella melitensis* is more like *Brucella abortus* isolated from swine. Such results seem rather inconsistent. Dr Simpson mentioned the fact that he had found a positive blood serum on five women frequently aborting. I have examined tissues from about 50 cases of abortion and I have isolated *Brucella abortus* from one case. Kristensen has reported the recovery of *Brucella abortus* from a cystic ovary. It is interesting to note that the reports state that *Brucella melitensis* infection is more common in children than in adults. In this country *Brucella abortus* infection seems to be more prevalent in the adult.

Dr D Schuyler Pulford—I would like to mention an experience I had with treatment of undulant fever. We have had seven cases of this disease in the Woodland Clinic. One had an associated periorbitis to a quite marked degree. We had tried various treatments in other cases without avail so in this one we decided to give the patient a course of amidoxyl benzoate as used in the treatment of arthritis. With this treatment the periorbitis disappeared. I think it worthy of consideration that you might cure this infection by the administration of a course of amidoxyl benzoate.

Dr E E Mudge—There is just one question that came to my mind. We have noted the marked difference in every instance between the papers given by Dr Giordano and the discussion of Dr Simpson and others. I wonder if it would be possible that the use of mechanical milkers on the one hand, and manual milkers on the other might explain the 90 per cent instance.

Dr Walter M Simpson—Contagious abortion of cattle is a very bad term. In the first place, thousands of cattle that are not pregnant acquire *Brucella abortus* infection. In the second place, bulls have it, in the third place many pregnant cows suffering from the infection do not abort. The question arises as to what constitutes an abortion free herd. Merely to have no cattle in the herd that have a history of abortion is not enough. By use of the agglutination test one can determine which animals are reactors. But we know that there are instances in which the organism has been recovered from the milk and no antiabortus agglutinins were present in the serum. It is erroneous to say that the etiology of undulant fever is unknown. The etiology is most certainly known. The proof is conclusive that most of the cases of undulant fever in this country are the result of the ingestion of raw milk of unpasteurized dairy products. The important consideration is that a human being is sick as a result of an infection with some organism of the *Brucella* group acquired from raw milk. Just which one of the sub groups is the actual invader is not so important from the clinical point of view. We must not miss the forest because one tree obstructs our view.

Dr A V Hardy—I am sure that you all understand that we did carefully consider the possibility of milk being the mode of spread. The packing houses in Iowa are however in cities of over thirty thousand and in these cities particularly in Sioux City practically all of the milk, 80 to 90 per cent is pasteurized. We have found in our packing house workers that as a whole they are not heavy milk users. We have gone into that very carefully and the evidence seems almost conclusive, that milk played practically no part in the occurrence of these infections.

Dr Charles M Carpenter—Most of the infection in milk rises with the cream. Cream and butter are infected more heavily than raw milk. *Brucella abortus* can live in butter for four months when stored at eight degrees C. We consider that raw milk is the chief source of infection but one may become infected through butter or cream.

may lead to erroneous ideas of the source of the virus. A series of animal experiments were carried out with this in mind.

The rabbit seemed to be best suited for the work since rabies rarely ever develops except as the paralytic form. Also they seldom show drooling of saliva, nor do they lick themselves to the extent shown by other animals. The time of pregnancy is short, and inoculation of the animal can be closely controlled with the time of delivery. Also virus which will produce rabies in the rabbit should be virulent for other rabbits if present in the milk, while there might be a lessened virulence for other animals.

Three full-grown does were bled and four days before expected delivery, they were injected subdurally with brain emulsion from two rabid dogs. The brain substance from each dog had shown Negri bodies, and had also produced experimental rabies in rabbits. The animals were injected late to avoid possible placental transmission⁹ which has been reported in animals. Also that the young might be almost weaned by the time symptoms set in.

The litters were allowed to suckle the doe almost to the time of her death. Restraint was required as a rule to permit the young to approach the breasts after symptoms ensued. With the onset of symptoms milk was also expressed from the several breasts daily, mixed and injected into the masseter muscle¹⁰ of two half-grown rabbits. The above brain emulsion injected into the masseter muscle of control rabbits produced experimental rabies in twelve to sixteen days.

Neither rabbits of the litters nor those injected with milk from those that died, have shown symptoms of rabies. Nor has search for Negri bodies or subdural inoculation of brain tissue from these animals that died, been successful. Autopsy has with few exceptions shown a definite cause for death. Milk injections did not produce an abscess in any case. The remaining animals have grown, and are healthy after three months.

The results obtained in this small series of animals seem to exclude transmission of rabies virus through breast milk. This same view has been expressed by Heinemann,¹¹ Kenle¹² and Nicolas.¹³ But virus may be present from saliva. If present, in whatever form, it may potentially produce the disease. Gastric juice destroys the virus in approximately five hours, and it is highly improbable that intact mucosa of the gastrointestinal tract will permit it to pass into the tissues. Freezing is without effect, but heat will kill the virus. There is some disagreement on the thermal death point, Marx¹⁴ reports forty minutes at 58° C, and van den Hoven van Genderen¹⁵ shows that brain emulsions heated from 50° to 65° C for fifteen minutes are still virulent.

All are agreed that boiling destroys the virus in a very few minutes. Drying is detrimental, but the time interval before virulence is lost is dependent on several factors, rapidity of desiccation, temperature, presence of oxygen and light, all influence the results. Oxygen seems especially detrimental, as virus in saliva drying in air and sunlight may be destroyed in fourteen hours,¹⁶ while protected from oxygen, the virulence persists much longer. In milk, virus probably remains unchanged a long time, although no definite data has apparently been obtained. It is known that in the presence of moisture and

especially at ice box temperature virus keeps for weeks. Also virus is not destroyed early in putrefaction.¹⁷

The question has arisen of the efficacy of pasteurization of milk if rabies should be present. Regulations in most States require a temperature of at least 61° C for thirty minutes. Experimental data is not certain that this is sufficient to destroy rabies virus.

Evidence is not clear that rabies can be transmitted through milk, except from extraneous contamination. Further evidence shows with rare exception that ingestion of known virus does not result in rabies in experimental animals and the same results should hold for man. The question is whether rabies vaccine should be given in these cases.

There is the economic problem, for it would be a heavy expense to treat all individuals in an institution of any size or even a small group of people who might partake of milk from a rabid cow. But treatment is indicated, if any risk is present. On this authorities are inclined to be indefinite. Should some degree of risk be present it is doubtful if it is as great as the development of posttreatment paralysis. This complication is not seen as often now, since the various modifications of the Pasteur treatment have apparently lowered the incidence. But it is still a problem in all parts of the world where any great number of the treatments are given and the risk should be considered, as a long convalescence, or even death may ensue. Should a group of individuals be subjected to a definite risk however small to gain protection against an improbable infection?

SUMMARY

The general increase in rabid animals will bring up the possible transmission of rabies through milk more often in the future. Literature is conflicting on possibilities of such infection and advice is indefinite on the necessity of treatment.

A small series of rabbits either injected with milk from rabid does or nursing these does did not develop rabies.

Data does not seem to justify antirabic treatment for individuals who have ingested milk from a rabid cow.

Pasteurization as ordinarily conducted may not destroy rabies virus present.

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MALIGNANT NEOPLASMS OF THE TESTIS*

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MALIGNANT neoplasms of the testis, while not rare, are not common. About 700 cases have been recorded in literature. Several times that number have probably been actually observed. On two fairly large surgical services I have found that about three cases a year is their average. The present status of our knowledge on the subject is more or less chaotic, and yet, it is rapidly improving and is considerably more satisfactory than only a few years ago when nearly all of the surgeons as well as some pathologists considered nearly all of these tumors as being sarcomas.

The present opinions on the subject are well known and can readily be divided into two groups. (1) the following, headed by Ewing¹ who believes that all malignant tumors of the testis are teratomas, and (2), the group composed principally of Chevassu,² Schultz and Eisendrath,³ Southam and Linell,⁴ Tannel,⁵ Bell⁶ and others who take the position that, while teratomas constitute a large group of these tumors, there is an equally large and important group of tumors composed of homologous epithelial cells and presenting no evidence of teratomatous elements. These pure epithelial tumors are composed of cells which are usually described as being large spherical cells with clear cytoplasm and large vesicular nuclei. Because of their resemblance to the epithelial cells lining the seminiferous tubules, those concerned with spermatogenesis, these neoplasms have been called seminomas or spermatocytomas.

Ewing's opposition to the existence of such a growth has been that they were only apparently homologous, an instance either of failure to examine the tumor with sufficient care or of one type of cell proliferating to the nearly or entirely complete obliteration of other cells originally present.

Not long ago such a view was considered by many absurd, requiring a

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ply of imagination and to say the least extreme open mindedness. Today one can appreciate the wisdom of Ewing's position and the fearlessness necessary to be the first to express such a viewpoint because the theory is necessarily somewhat vague and the proof not easily demonstrated.

The basis of this paper is a review of 32 cases collected during the past ten years. First, merely the existing slides were reviewed. Later from six to fifteen more blocks were taken from each specimen. Then in each case the second diagnosis was compared with the one originally made. The original diagnoses were interesting and amusing ranging from spermatocytoma to teratoma and including lymphoma, round cell sarcoma, various types of carcinoma, and malignant adenomas.

It was interesting to find that on reviewing the original slides on the basis of an accepted and apparently reasonable classification (the most rea-

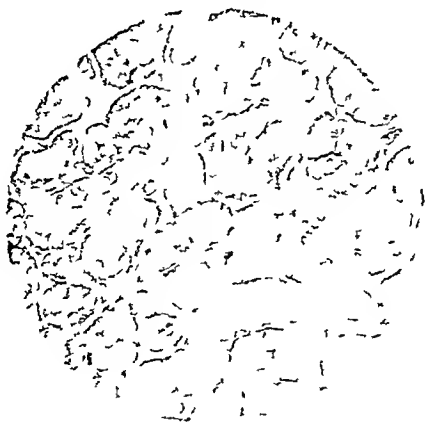


Fig. 1—E. ntial n n l t t t

sonable I thought at the time) there were exactly six teratomas and sixteen spermatocytomas or pure carcinomas (homologous (epithelial tumors). But when the slides were examined from the six to fifteen additional blocks from each specimen it was necessary to remove six cases from the spermatocytoma group into the teratoma group leaving twenty-two teratomas and ten tumors which revealed no heterologous elements. This proved the correctness of Ewing's contention. Sufficiently careful examination had detected heterologous elements in all but ten cases where probably the number of sections was still insufficient or where there was an extensive overgrowth of one type of cell. The important point is that by the examination of a large number of slides it was possible to make a diagnosis of teratoma where a small number of sections from each specimen furnished insufficient evidence to make such a diagnosis.

Teratomas are not any too thoroughly understood and the teratomas of

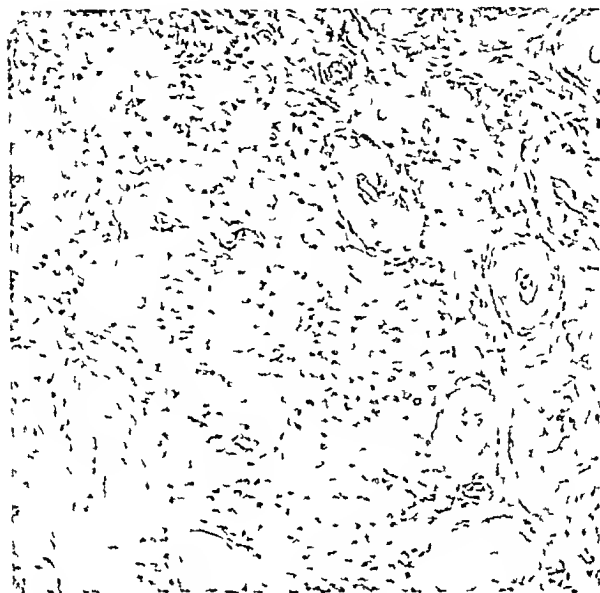


Fig 2—A small malignant area in undescended testicle



Fig 3—Typical newgrowth area composed of germinal cells

the testis are a special problem. At a very early stage in the formation of the mesonephros, a narrow strip of mesothelium extending along the medial surface becomes thicker and the cells become arranged in several layers. This is the germinal epithelium which is composed of two kinds of cells: (a) small cuboidal cells with rather intensely staining cytoplasm which become interstitial cells, and (b) the large spherical cells with clear cytoplasm and larger vesicular nuclei referred to above and which are the sex cells, destined, in the male, to give rise to spermatozoa.

The germinal cells are important in the histogenesis of malignant tumors of the testis. Their appearance is characteristic; they are easily recognizable and they were present in every case in this series. They are epithelial cells derived from the mesoderm. It is easy to see how the term 'embryonal carcinoma'



Fig. 4—Newgrowth area with pattern suggestive of normal testis

noma' came into usage because in spite of the bidermal or tridermal nature of the tumor these characteristic epithelial cells are always or usually present. The presence of cartilage in newgrowths of the ovary or testis is usually interpreted as evidence of a mixed tumor and yet cartilage with these typical germinal cells would strictly speaking be only a monodermal tumor, all cells being derived from the mesoderm; however I have never found cartilage in these tumors in the absence of both ectodermal and endodermal derivatives.

These typical cells which have served to confuse and complicate the interpretation of these teratomas have quite a wide range of variation of morphology. Schultz and Eisendrath³ believe they can recognize the different stages of spermatogenesis which these cells represent but that is doubtful. Variation in the morphology of cells composing tumors of any organ is common. Appreciation on the part of the microscopist of the variation in cell arrangement is most important when attempting to make a diagnosis of a

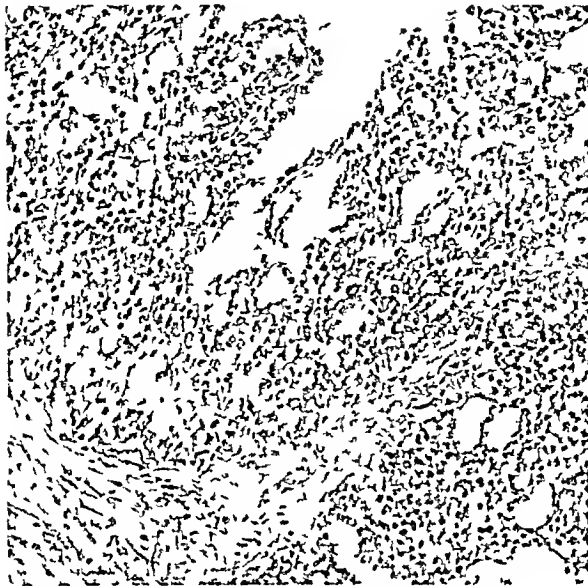


Fig 5—Germinal type cells with papillary arrangement

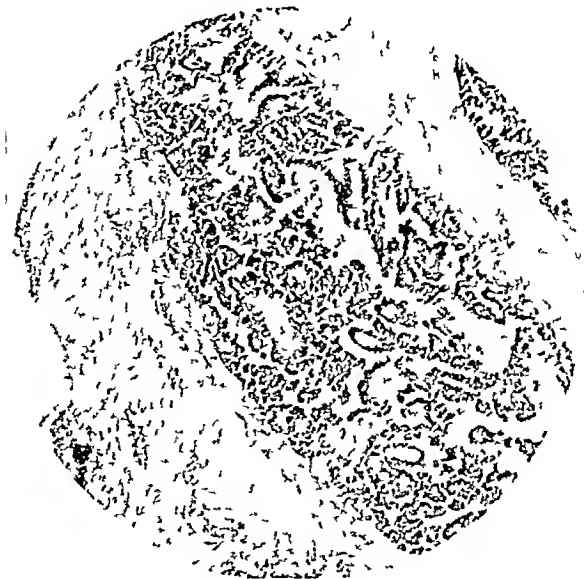


Fig 6—Teratoma of testis with definite papillary arrangement of epithelial cells

neoplasm of the testis. The cells frequently grow in a solid encephaloid formation with a marked tendency to necrosis (Figs 2 and 3). Again they assume an alveolar arrangement which suggests a definite relationship to the tubular epithelium, just as malignant cells elsewhere bear a resemblance to the normal cells to which they are related (Fig 4). But unfortunately they also assume a bizarre alveolar arrangement, in no way suggestive of normal testicle sometimes with a marked papillomatous arrangement (Figs 5 and 6). Most confusing of all is when these cells become so atypical in morphology and arrangement that they cannot be distinguished from highly malignant cells of endodermal derivation (Fig 7). I believe it is often impossible to say definitely when examining these tumors microscopically whether certain cells are germinal or endodermal in derivation.



Fig 7—Newgrowth cells of doubtful derivation.

The malignancy of these tumors is either wholly or in part due to the germinal type cells and metastases usually consist of these cells. However the cells of endodermal derivation can and do become highly malignant but the malignancy of tumors of the testis is invariably due to epithelial cells.

The prevalence of germinal epithelial cells in teratomas of the testis has been explained by Ewing as the result of a one-sided development. That hardly accounts for the uniformly consistent presence of these cells. Why should differentiation proceed so unvaryingly in this one direction?

Other authors¹¹ have suggested another explanation which, on the surface at least sounds quite reasonable. The sex cells at an early stage are totipotent, i.e., capable of producing any order of cell in the body. Therefore a mixed tumor might arise from misplaced germ cells forming rests which proliferated later with one type of cell possibly becoming the most aggressive, resulting in the formation of an apparently homologous tumor.

It should be remembered that small cysts frequently in the capsule must



Fig 8 —Newgrowth cells extending through adjacent seminiferous tubules

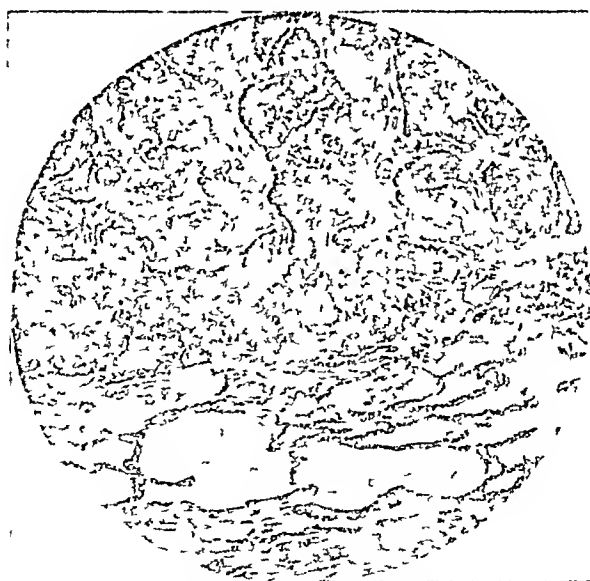


Fig 9 —Typical metastatic area in lung



Fig 10—Newgrowth cells resembling interstitial epithelial cells of testis

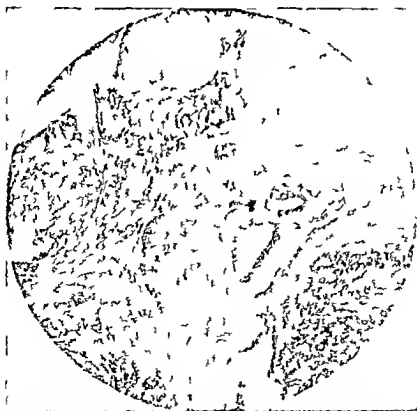


Fig 11—Different types of epithelium associated with cartilage in a teratoma of the testis

not be confused with endodermal derivatives but are probably müllerian duct derivatives

From a careful study of the slides from these cases it seems apparent that the newgrowth cells extend to adjacent testicular tissue by way of the seminiferous tubules. It is not difficult to find areas in which the newgrowth cells fill the tubules for a full low power field or more beyond the growth in the intertubular stroma (Fig 8)

The material studied furnished evidence that not only the tubular epithelium but also the interstitial epithelium were closely related to the newgrowth. This is not surprising when the embryology of the testis is considered. In one case there were extensive metastatic areas in the liver, lungs, and retroperitoneal lymph nodes (Fig 9), but numerous sections of the testis showed only one suspicious area consisting of hyperplastic cells resembling interstitial epithelium and histologically not malignant.

In another case the metastatic areas in the liver consisted largely of cells which closely resembled the interstitial epithelial cells of the testis (Fig 10). A paper is now being prepared to present the theory that a constant transition takes place between the intertubular cells and the interstitial epithelium in the same way that this interrelationship has been claimed to exist between acinar and islet cells of the pancreas by Otani,⁷ between the parenchymal and reticular cells of the thymus by Gottesman and Jaffe,⁸ between the liver rod cells and bile duct epithelium by MacCallum⁹ and between the acinar and interstitial cells of the thyroid by Hertzler.¹⁰ According to some embryologists the interstitial epithelial cells are derived from sex cells and according to others they come from the interstitial connective tissue.

SUMMARY AND CONCLUSIONS

1 The histopathology of tumors of the testis is often confusing and frequently misleading so that an accurate classification is difficult to arrive at.

2 The fact that, by the examination of additional sections in each of the thirty-two tumors in this series it was possible to transpose six cases from the homologous tumor group to the mixed tumor group, is valuable proof of the correctness of Ewing's position.

3 However, Ewing's explanation that the almost pure epithelial nature of these tumors is due to a one-sided development of a teratoma does not appear as reasonable as to say that these tumors arise from very young sex cells which are still totipotent and are therefore capable of giving rise to heterologous elements. Additional proof for such a view lies in the established fact that mixed tumors of the testis are more malignant than the so-called spermatocytomas which could be construed to mean that mixed tumors are derived from very young germ cells, capable of producing ectodermal and endodermal tissue, whereas the homologous nature of some of these tumors may be due to the misplaced germ cells taking on newgrowth characteristics after their totipotent limitation had been reached.

4 While I think that the proper conception of these tumors is that they are all mixed tumors, I am not sure teratoma is a good term. They do not possess many of the characteristics of teratomas. It is difficult to think of

them in a parasitic sense and besides they are so predominatingly epithelial in composition. Possibly embryonal carcinoma is preferable.

5. Definite proof cannot be offered to bring about the sudden acceptance of the view that malignant tumors of the testis are universally mixed tumors yet pathologists who in their daily routine work considering the subject carefully will doubtless be slowly but surely converted to this view.

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2201 JEFFERSON AVENUE E.

RETICULOCYTES THEIR IDENTIFICATION AND SIGNIFICANCE*

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THE red corpuseles of the blood have been extensively investigated. They are free in the blood stream and are easily available for study. They are unique in that they normally function after the loss of the nucleus. Key¹ says mature erythrocytes have lost their cytoplasm and are not living cells. Hariot² observes that the normal mature erythrocytes have no measurable oxygen consumption. The nature of the internal structure has received considerable attention. The erythrocyte may be considered as a balloon containing a stroma and a functioning substance as a relatively fluid colloid. The hemoglobin part may be in the state of a hydrophilic gel. The covering consists of a lipid and protein stroma. Ultramicroscopic examination of the erythrocyte fails to reveal anything as to its structure. The number of erythrocytes shows considerable variation as does the hemoglobin. The controversy as to the single or multiple origin of blood cells need not concern us, except that Ehrlich³ advanced the polyphyletic theory while the monophyletic theory is advocated by Pappenheim,⁴ Maximow,⁵ Danschakoff.⁶ Sabin quoted by Woollard,⁷ more recently, has given a detailed account of the morphologic changes that occur in the various cellular forms that precede the formation of the red corpusele in the chick. From the common mesenchyme cell is derived the angioblast. These basophilic cells form synektial masses, sprout to form plexuses, and form primitive blood vessels and primitive blood cells. Red blood cells actually arise from the endothelium and the primitive angioblast. We have no knowledge of the factors which induce the primitive mesenchyme cells to form the endothelium of the first capillaries.

The youngest representative of the red cell, according to Woollard,⁷ is a fairly large round cell with only a faint trace of hemoglobin in the deeply basophilic cytoplasm, with a large vesicular nucleus. They show large rods (mitochondria?) by super vital staining with Janus green B. This cell and its immediate offspring may be considered as megaloblasts. They are an indication of active erythropoiesis and are seldom seen in normal bone marrow. They are followed by the erythroblast, also showing rods, more hemoglobin, and a denser nucleus. The nucleus becomes more pyknotic and is finally extruded. The cytoplasm becomes apparently homogeneous and the granules and rods disappear entirely. We now have the adult (senile) erythrocyte ready to begin its life's work. How long this cell continues to function is not definitely known. It has been discussed by Rous.⁸ It is estimated that one-fifteenth of the erythrocytes of the body are destroyed daily. The average life expectancy, therefore, is about fifteen days. Quincke estimates it at thirty days. Ashby⁹ says, "Estimates vary from two to four weeks." The useless cells are quickly removed from the circulation, but the fate of the erythrocytes is, by no means, a settled question (Rous⁸).

*Read by title before the Eighth Annual Convention of the American Society of Clinical Pathologists, Portland, Oregon, July 5, 6 and 8, 1929.

Vital staining consists in mixing a drop of freshly drawn blood with a bit of stain and observing the blood before it is dried Ehrlich,⁸ using methylene blue in this way, found in the cytoplasm of some of the cells a bluish network Pappenheim,¹⁰ using neutral red, observed granules in anemias Ceasaris Demel, using cresyl violet, observed granules, filaments, and networks In Europe these cells were called "granulo reticulo filamentous cells" In America they have been called "reticulated cells" and Krumhhaar¹¹ suggested the word "reticulocyte" and regarded an increase in the number of these cells as a "reticulosis" Adult human blood contains from 0.1 per cent to 1 per cent, while in infants, during the first week of life, the reticulocytes show a much higher percentage At the end of the first week, the blood begins to resemble the adult picture Physiologically all are agreed that the reticulocytes are young, growing red cells

Key² has studied reticulosis in considerable detail, and his results are briefly given When a drop of blood is mixed with brilliant cresyl blue, the reticulum is quickly stained a purple A moss like wreath is the usual form but all gradations, ranging from cells containing a few basophilic granules or fragments, through rather coarse networks and loosely constructed wreaths can be seen in the same blood In addition to these cells one often sees cells which stain diffusely In nucleated red cells of embryonic blood, the reticular network surrounds the nucleus An occasional nucleated red blood cell is found in which no reticular substance can be seen Azur II stains the reticulum blue thionin stains the reticulum blue methyl green does not stain the reticulum It can also be stained with many other basic dyes Janus green B, used extensively as a specific (?) supravital stain for mitochondria will stain certain of the erythrocytes a faint diffuse green color A variable number of small green granules and rods appear connected by delicate green strands so that a definite reticulum is formed This green color gradually fades leaving the reticulum as a refractile network The percentage of erythrocytes containing reticulum in a preparation stained with Janus green B corresponds closely with the percentage of reticulated cells in a preparation of the same blood stained with brilliant cresyl blue Cowdry¹² was able to stain mitochondria in lymphocytes with a solution of 1:500,000 Janus green B In order to stain the reticulum in the erythrocytes, much stronger solutions are necessary A concentration of 1:6000 was found satisfactory

Air dried smears of anemic blood, stained with any of the stains which stain reticulum supravitaly fail to stain the reticular substance in wreath like form, but as basophilic fragments and granules and there is no diffuse staining

In true polychromatophilia the staining is diffuse and no granules or fragments are seen In Wright's method after alcohol fixation and staining the picture is one of polychromatophilia but if the Wright stain be first allowed to dry on the slide, and the staining is done by the supravital method a definite basophilic reticulum appears

It has long been known that a basic staining substance is present in certain erythrocytes and that it can be demonstrated in the form of polychromatophilia, punctate basophilia, or reticulum by appropriate staining

methods It is quite generally believed that these basophilic forms are young erythrocytes In the circulating blood of normal adult human beings no polychromatophilia or punctate basophilia and not over one per cent of reticulated red cells are found Basophilic erythrocytes are commonly found in adult bone marrow In anemic adults the percentage of basophilic erythrocytes is roughly proportional to the activity of the bone marrow

Hawes¹³ concludes that polychromatophilia stippling, and reticulation are all different manifestations of the same process

The addition of oxalate to the blood does not seem to affect the reticulum of the erythrocytes, and the reticulum can still be stained

Schilling-Toigau¹⁴ states that the reticulated erythrocytes, treated with very dilute alkalis and then stained supravitaly, give pictures resembling punctate basophilia and he considers this as a transition stage between reticulation and polychromatophilia

If we consider erythrocytes which contain basophilic substance as young erythrocytes, these cells are slightly larger than mature cells (Hawes¹³) and apparently contain a lower percentage of hemoglobin Reticulated cells exhibit a tendency to agglutinate The specific gravity of reticulated cells is slightly lower than that of normal erythrocytes The reticular substance is not soluble in water or in sodium chloride solution, and in blood which is laked in distilled water, the refractile granules are not dissolved and can be seen stained in the usual manner (Key¹) The reticular substance is not soluble in ethyl or methyl alcohol, and upon the addition of 5 per cent acetic acid, reticular substance can still be seen in a fragmented form The reticular substance appears as refractive granules in solutions of various acids, and is insoluble

That the presence of basophilic substance in erythrocytes is evidence of youth is indicated by the fact that basophilia and reticulation in erythrocytes is increased in states in which blood formation is stimulated, and decreased in states in which it is inhibited, not only in clinical states, but also in experimental conditions

Basophilic cells are present in increased numbers in embryonic blood, and their percentage progressively decreases as the embryo approaches term They are also present in large numbers in bone marrow and in embryonic livers where blood formation is in progress and the basophilic substance is demonstrable in nucleated red cells, and can be seen to remain in the cell as the nucleus is being extruded

According to Key,¹ the basophilic substance, which he considers a better term for reticulum, does not appear in any other cells of the body In no other cell is the protoplasm replaced by hemoglobin, and in no other cell is the nucleus lost

The state in which this basophilic substance exists in the unaltered erythrocyte is unknown Apparently either the diffuse polychromatophilia or the reticular net is due to the technique used

The reticular substance may be distinguished from the nucleus by differential staining, and it is believed that the reticular substance is of cytoplasmic origin It may be a remnant of the protoplasm which is ultimately replaced by the hemoglobin

METHODS OF STAINING

There are a number of methods in use for demonstrating the reticular substance of erythrocytes. Brilliant cresyl blue has been extensively employed. The blood can be added to a solution containing about 0.2 per cent of brilliant cresyl blue and 1 per cent neutral potassium oxalate in 0.85 per cent sodium chloride solution, allow it to stand from ten to twenty minutes, centrifuge to precipitate the corpuscles, transfer the sediment to a clean slide, air dry, and examine with the immersion lens. The percentage of reticulocytes can be calculated after examining at least three thousand cells for reticulation. An ocular micrometer disc, suitably ruled, greatly facilitates counting.

A method which is more frequently employed and which in our hands has given excellent results is one in which scrupulously clean covers or slides are used, the surface of which is covered with a thin layer of the dye. These are prepared by placing on the slide a drop of 0.5 per cent alcoholic solution of brilliant cresyl blue, spreading it and allowing it to dry in the air, or drying can be hastened by passing the slide through a flame. It is essential to avoid the collection of dust on the surface of such smears. A drop of blood is placed on the slide which is covered with the thin film of dye and a clean cover glass is dropped on. The preparation is ringed with vaseline. After allowing one minute for staining, the counting can proceed.

A modification of this method is to omit the ring of vaseline and after the lapse of one minute, raise the cover glass gently, spread the drop of blood with the cover, air dry, counterstain with Wright's method, and enumerate as before mentioned. Or a cover glass filmed with the dye can be used in a similar manner. In the wet preparations ringed with vaseline, it is possible to enumerate blood platelets at the same time as they are tinged a faint lavender color.

The number of reticulocytes is usually reported in percentage or the total number per cubic millimeter can be reported. By so reporting we can quickly note the total number of reticulocytes as compared with the average number found in normal blood. Furthermore, valuable information is obtained by comparing the total number of reticulocytes present in the blood at a given time with the total number of cells present in the blood of the patient. It is also of value to compare the total number of reticulocytes per cubic millimeter in successive counts.

CLINICAL SIGNIFICANCE OF RETICULATED CELLS

The reticulocyte is universally considered to be an immature red blood cell, but the nature and meaning of the reticular substance, the relation which it bears to the nucleus, and the mode by which the nucleus is lost to the cell are still disputed. The presence of normoblasts in the normal red marrow and the occurrence of reticulocytes in small numbers suggests that in all probability they are the normal stages in the evolution of the anuclear cell. The characteristics of the nuclear material and the characteristics of the reticular or basophilic substance indicate that the reticular substance is probably of

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The addition of ovalate to the blood does not seem to affect the reticulum of the erythrocytes, and the reticulum can still be stained

Schilling-Toigau¹⁴ states that the reticulated erythrocytes, treated with very dilute alkalis, and then stained supravitaly give pictures resembling punctate basophilia and he considers this as a transition stage between reticulation and polychromatophilia

If we consider erythrocytes which contain basophilic substance as young erythrocytes, these cells are slightly larger than mature cells (Haves¹³) and apparently contain a lower percentage of hemoglobin Reticulated cells exhibit a tendency to agglutinate The specific gravity of reticulated cells is slightly lower than that of normal erythrocytes The reticular substance is not soluble in water or in sodium chloride solution and in blood which is laked in distilled water, the refractile granules are not dissolved and can be seen stained in the usual manner (Key¹) The reticular substance is not soluble in ethyl or methyl alcohol and upon the addition of 5 per cent acetic acid, reticular substance can still be seen in a fragmented form The reticular substance appears as refractive granules in solutions of various acids, and is insoluble

That the presence of basophilic substance in erythrocytes is evidence of youth is indicated by the fact that basophilia and reticulation in erythrocytes is increased in states in which blood formation is stimulated, and decreased in states in which it is inhibited, not only in clinical states, but also in experimental conditions

Basophilic cells are present in increased numbers in embryonic blood, and their percentage progressively decreases as the embryo approaches term They are also present in large numbers in bone marrow and in embryonic livers where blood formation is in progress, and the basophilic substance is demonstrable in nucleated red cells, and can be seen to remain in the cell as the nucleus is being extruded

According to Key,¹ the basophilic substance, which he considers a better term for reticulum, does not appear in any other cells of the body In no other cell is the protoplasm replaced by hemoglobin, and in no other cell is the nucleus lost

The state in which this basophilic substance exists in the unaltered erythrocyte is unknown Apparently either the diffuse polychromatophilia or the reticular net is due to the technique used

The reticular substance may be distinguished from the nucleus by differential staining, and it is believed that the reticular substance is of cytoplasmic origin It may be a remnant of the protoplasm which is ultimately replaced by the hemoglobin

following a transfusion. Not uncommonly he found that when a remission was unlikely, because of low reticulocyte count, transfusion caused apparently the necessary stimulating effect and there followed a marked increase in the reticulocyte count and a remission occurred.

He also noted that polychromatophilia, an accepted evidence of regeneration in the fixed preparation, runs parallel with the reticulation of the unfixed smear, at times.

In a few cases of aplastic anemia observed, a condition representing a rapid failure in bone marrow growth, the reticulocyte count is found to be almost nil and remained so until death.

In purpura hemorrhagica, in which there is a failure in the platelet growth resulting in hemorrhage and anemia, it was observed that when the reticulocyte count was low and remained so, the patients continued to bleed and finally died. When the reticulocytes increased, the platelets increased and the patient recovered.

He also found that in congenital hemolytic anemia, marked reticulosis is pathognomonic, differentiating that disease from other anemias associated with large spleens.

In a report by W. W. Duke,¹⁸ aplastic anemia, originally described by Ehrlich, is caused primarily by aplasia of the bone marrow. As a sequence the rate of blood regeneration is reduced and anemia inevitably follows. The symptoms displayed by a patient with aplastic anemia depend on which of the formed elements of the blood are most markedly reduced. If the red cell reduction dominates the situation, anemia is the prominent symptom. If the thrombocytic element is the most markedly involved, the prominent symptoms are hemorrhage or purpura hemorrhagica. It is generally accepted that the agent that injures the marrow also tends to destroy the formed elements in the blood itself. This may give a blood picture similar to that of pernicious anemia.

The type of severe anemia generally called "aplastic" anemia because the bone marrow in such a case makes little or no effort to regenerate the blood cells is at times caused by known agents, and is then known as "secondary aplastic" anemia, when the cause is unknown the term "idiopathic aplastic" anemia is applied.

The blood picture reflects the status of the bone marrow as seen at autopsy, and Rennie believes it due to a congenitally weak bone marrow, which at an early age becomes unable to carry on the function of blood formation.

Murphy and MacEachern¹⁹ discussed the differentiation between aplastic and pernicious anemia. Ordinarily the diseases that are confused with aplastic anemia are pernicious anemia, purpura hemorrhagica, and myelophthisic anemia. Aplastic anemia with secondary purpura may be difficult to differentiate from idiopathic purpura hemorrhagica. Minot²⁰ believes that there may be a relation between the two conditions and describes what he calls intermediate cases. In some cases (purpura hemorrhagica) the bone marrow cannot respond to the stimulus received by hemorrhage as it should, and the white and red elements of the bone marrow may have been depressed.

Minot also claims that the aplasia may involve the platelets markedly, white cell elements slightly, the red cell not at all, and regenerative forms may be numerous

In idiopathic purpura hemorrhagica the white cell count is usually increased and the red cell count indicates the normal response on the part of the red cell centers to furnish erythrocytes to the blood stream which has lost many cells through hemorrhage resulting from a thrombopenia. A decreased platelet count is first noted and a decrease in the red blood corpuscles and white blood corpuscles follows. In true idiopathic aplastic anemia red blood corpuscles and white blood corpuscles decrease first and a platelet decrease follows, anemia before hemorrhage

According to Duke²¹ the reticulocyte count is a very important means of observing whether or not a patient with pernicious anemia will respond to a liver diet. Counts should be made daily in order to avoid missing the crisis which should reach its maximum in about a week and may be as high as 30 per cent reticulocytes. Reticulocyte crisis is apparently explained as suggested by Peabody, who pointed out that in the relapsing stage of pernicious anemia the bone marrow is packed with red cells of immature megaloblastic type which appear incapable of completing their development. If thrown into the circulation in this state, they are rapidly destroyed. He suggests that the action of the liver is to supply a stimulus enabling these cells to reach maturity. The reticulocyte crisis may be looked upon as representing an act of catharsis on the part of the bone marrow after which cell production proceeds in a normal manner.

In aplastic anemia if the platelets fall below 20,000 to 60,000, purpura hemorrhagica appears and may give rise to a profuse hemorrhage from the nose, gums, gastrointestinal tract, bruises, etc. Petechiae follow slight injuries. With this symptom complex goes the finding of normal coagulation, prolongation of the bleeding time, firm, but nonclotable clot, and petechiae. Duke finds that transfusions are useful in restoring the red cells and platelets which may be of several days' duration and also finds that diet is of less value than in pernicious anemia.

Doan²² suggests that the suppression of red blood cells and hemoglobin formation are due, not to lack of substances in the liver, but to an excess of some inhibiting substance. It is suggested that the factor of inhibition or suppression of blood formation in the megaloblastic stage may be a beneficial factor in liver diet treatment of pernicious anemia.

Ashford²³ says that the anemia of sprue yields a shower of reticulocytes when liver extract is administered, unless the bone marrow is aplastic. Isaacs, Sturgis, and Smith²⁴ state that the type of young or new red blood cell delivered into the peripheral circulation depends on the stage to which the erythroblastic tissue in the bone marrow has developed. When the bulk of the cells are in the nucleated (megaloblastic) stage, the first evidence of stimulation by liver therapy is increased development to the next stage (reticulocyte). When the bulk of the cells in the bone marrow are in the latter stage, stimulation is followed by the increased production of mature cells. Many other writers, Middleton, W. S.,²⁵ Whipple, G. H.,²⁶ West, R., and Nichols, E. G.,²⁷

and Means and Richardson² consider a reticular response as evidence of the value of liver therapy. Light is thrown on the relative age of a given erythrocyte by the appearance of its reticulated substance (Cassirer Demel). While the percentage of reticulocytes in the circulation is raised whenever the new blood cells are being formed in increased numbers it by no means follows that the percentage of reticulocytes is commensurate with the degree of anemia. The response depends on the condition of the bone marrow, whether it is healthy or "damaged." It is much more marked in hemolytic jaundice than in pernicious anemia.

Berglund, at a meeting of the American Association of Physicians held at Washington, D. C. in May, 1928, read a paper on the use of fetal liver in anemias. At the Ohio State University Hospital there is a patient who was admitted in April, 1927, suffering from idiopathic aplastic anemia. Until August 1928 this patient was intensively treated with liver and liver extract. During this period he had frequent hemorrhages and in spite of numerous blood transfusions, showed a persistently low blood count. In August, 1928 fetal liver given raw and in quantities varying from 600 to 800 grams daily was employed with rather striking results. The reticulocytes previously absent, appeared in the blood stream during September, and by December 1928 his blood showed 10 per cent reticulocytes. Since that time there has been a gradual decline in the percentage of reticulocytes. During April 1929, the red cells reached a total of 3 300 000 while at the beginning of the fetal liver therapy the total number of red cells was only 1 300 000. The hemorrhages are much less frequent and only occasional blood transfusions have been indicated. This case was reported by Upham and Nelson *Fetal Liver Feedings in Aplastic Anemia*, at a meeting of the Missouri State Medical Association in May 1929 and shortly will be published.

SUMMARY

Reticulocytes are immature erythrocytes containing within the cytoplasm, a basophilic substance, which, by means of vital staining can be demonstrated in the form of a reticulum.

Reticular substance is not present in the adult erythrocytes.

The addition of oxalate to the blood does not interfere with the action of dyes which stain the reticulum.

The most suitable vital stains for reticulum are brilliant cresyl blue, or a suitable methylene blue.

With the introduction of liver therapy in pernicious anemias, the enumeration of reticulocytes is a valuable procedure. Inasmuch as it is a satisfactory method of noting the results of treatment reticulocyte counts should be made daily to determine whether a given case will respond to liver therapy, and in order to avoid missing the reticulocyte crisis.

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THE DETERMINATION OF HEMOGLOBIN WITH THE PHOTOELECTROMETER*†

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A YEAR ago we reported a new method for the estimation of hemoglobin. The apparatus as described has been modified and simplified during the last year. A detailed description of these changes will be presented in another paper.³ We also propose the name photoelectrometer for the apparatus. Briefly, we may state that the chief changes consist in the substitution of a micro ammeter in place of the galvanometer as the measuring device, and the introduction of one stage of amplification using an ordinary vacuum tube. The housing has also been improved, making the instrument more portable and less liable to outside disturbances. Many of the mechanical improvements have been made by Mr. Dana Rogers, of the Section on Biophysics.

With the changes indicated we have spent many weeks in calibrating the apparatus for oxyhemoglobin and in making new curves and tables. An attempt has been made to standardize all procedures so that the instrument may be easily used for routine work. We shall not present protocols of our various series of experiments, but rather present briefly those steps that we now deem necessary for the proper use of this method. Besides the assembling of parts in the metal housing and the micro ammeter it is necessary to use batteries as follows: one 6 volt "A" battery, three blocks of 22.5 volt "B" battery, one 4.5 volt "C" battery, and one 1.5 volt "dry cell."

Some of the connections to these batteries are made permanently, and others must be opened whenever the apparatus is left at rest for any length of time.

PERMANENT CONNECTIONS

1. Three 22.5 volt "B" batteries are connected in series, thus making a 67.5 volt "B" battery.

2. The negative (-) terminal at the "B" battery is connected to the negative (-) terminal of the micro ammeter (Fig. 1).

Read before the Eighth Annual Convention of the American Society of Clinical Pathologists, Portland, Oregon, July 5, 6, and 8, 1923.

*Since the preparation of this paper we have found that Stanley P. Reimann of the Research Institute of the Lankenau Hospital, Philadelphia, has published a preliminary report on the photoelectric cell as a colorimeter. This preliminary report was made at the New York meeting of the Society for Experimental Biology and Medicine, April 21, 1926.

†Patent applications are pending which cover the use of photoelectric cells in the measurement of hemoglobin and the general principle of the applications of such methods with selective spectral filters to the determination of the amounts of unknown substances in solution both with and without various accessories such as one or more stages of amplification and various detecting devices. These applications for patent rights have been made in order to control the development, accuracy and general serviceability of such devices so that those who acquire apparatus involving the principles disclosed may secure satisfactory equipment. Assignment of any rights granted will be made to the American Society of Clinical Pathologists.

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3 Another lead from the negative (-) terminal of the "B" battery is connected to the positive (+) terminal of the 45 volt "C" battery through a resistance of approximately 3000 ohms

4 The positive (+) terminal of the 15 volt "dry cell" is connected to the binding post on the instrument marked "15 +" (Fig 2)

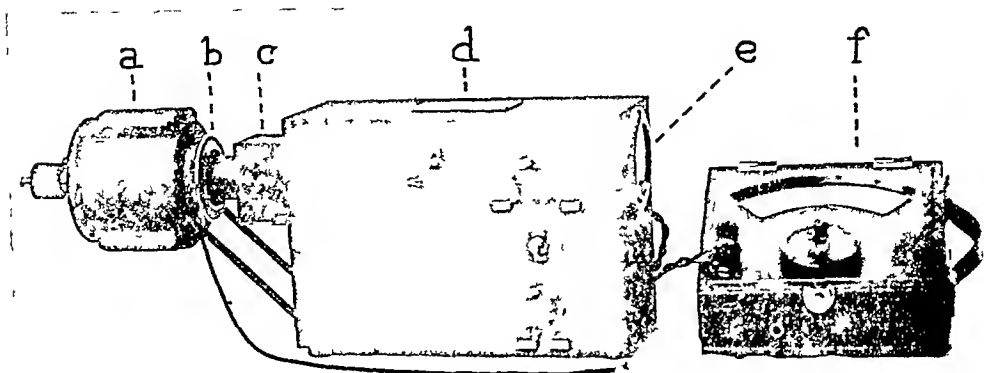


Fig 1—The photoelectrometer *a* Lamphouse and sources of illumination *b*, adjustable iris diaphragm *c* receptacle for holding diluted sample of blood and selective spectral filter *d* container for photoelectric cell and one stage of amplification *e* meter for reading current in illuminating circuit *f* micro-ammeter for reading current in circuit containing the photoelectric cell

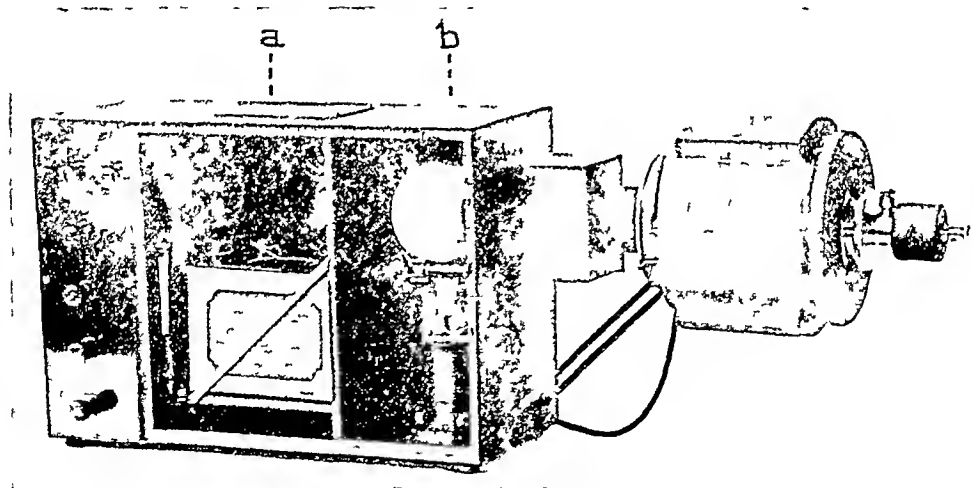


Fig 2—The photoelectrometer *a*, Showing the one stage of amplification with vacuum tube and *b* the photoelectric cell in position

5 The "A" battery terminals are now connected to the corresponding binding posts on the instrument ("A +" and "A -") (Fig 2)

6 The positive (+) terminal on the micro ammeter is connected to the terminal on the instrument marked "M+" (Figs 1 and 2)

The following connections are all permanent and are left undisturbed when the instrument is turned off. The shutter is always left closed while connections are made and a protective resistance controlled by the

lower knob on the front of the panel is turned to the position marked "in" Temporary connections are made only when the instrument is to be used and must always be made in the order described

TEMPORARY CONNECTIONS

7 The terminals from the "A" battery are connected to the terminals on the lamp house (Figs 1 and 2)

8 The filament rheostat (upper right hand knob) is turned "on" until the milliammeter on the end of the housing shows 250 milliamperes (0.25 amperes) (Fig 1)

9 The positive (+) terminal of the "B" battery is now connected to the binding post on the instrument marked B+ The micro ammeter will now register 70 to 80 micro amperes (Fig 2)

10 The negative (-) terminal of the 1.5 volt 'dry cell' is now connected to the binding post on the instrument marked "1.5 -" (Fig 2)

11 The positive (+) terminal of the 1.5 volt 'dry cell' is also connected to the negative (-) terminal of the 4.5 "C" battery When this is done, the needle on the micro ammeter will return nearly to zero (0)

12 The lower knob on the panel of the instrument which controls the protective resistance in series with the meter is now turned slowly toward the position marked "out," at the same time the position of the needle is carefully watched for any slight changes If there is any change in the position of the needle, the meter is brought to a zero reading by slowly adjusting the upper left hand knob on the panel The protective resistance should finally be turned all "out" (Fig 1)

13 A clean container (standard spectroscopic absorption cell) is placed in the compartment in the instrument and the shutter is opened to admit light from the lamp house to the photoelectric cell The deflection of the needle must indicate a current flow of 100 micro amperes and the iris diaphragm of the shutter must be adjusted until the needle shows exactly this deflection

The degree of deflection with an empty container must always be from zero (0) to 100 The shutter must always be closed immediately after a reading has been made

To disconnect the instrument operations 12, 11, 10, 9, 8 and 7 are performed in reverse order and in an opposite manner

DILUTING THE BLOOD

No essential change has been made in the method of preparing the diluted laked blood as described in our first paper Twenty cubic centimeters of the diluting fluid 0.1 per cent sodium carbonate is measured into a 50 cc centrifuge tube with a burette For convenience twelve centrifuge tubes are placed in a block constructed for this purpose It is necessary to use blood obtained by venipuncture, as it has not been found possible to measure accurately 0.1 cc of blood by using drops of blood from the ear It is therefore most convenient to make the dilution at the same time that the patient is being bled for tests on the serology or chemistry of the blood The blood may be pipetted from a small tube immediately after it has been placed

therein We have continued to use the pipette constructed for the Kahn test These pipettes are of 0.2 c.c. capacity and are graduated in thousandths of a cubic centimeter The blood is drawn just above the 0.1 c.c. mark, and the excess blood is wiped thoroughly from the outside of the pipette with a damp towel At the same time the column of blood is drawn down exactly to the 0.1 c.c. mark by placing the towel against the tip of the pipette It is then blown into the 20 c.c. of carbonate solution The diluent is drawn up into the pipette three or four times and expelled into the tube until the blood is washed from the pipette In doing this care must be taken to do all blow-

THE DETERMINATION OF HEMOGLOBIN WITH THE PHOTOELECTROMETER

MICRO AMMETER READING	HEMOGLOBIN, GM FOR EACH 100 C C	MICRO AMMETER READING	HEMOGLOBIN, GM FOR EACH 100 C C
30.0	23.58	55.5	11.20
30.5	23.10	56.0	11.05
31.0	22.75	56.5	10.85
31.5	22.5	57.0	10.70
32.0	22.15	57.5	10.60
32.5	21.80	58.0	10.40
33.0	21.50	58.5	10.25
33.5	21.25	59.0	10.10
34.0	20.85	59.5	9.90
34.5	20.65	60.0	9.75
35.0	20.30	60.5	9.65
35.5	20.00	61.0	9.50
36.0	19.65	61.5	9.35
36.5	19.40	62.0	9.20
37.0	19.20	62.5	9.00
37.5	18.90	63.0	8.85
38.0	18.65	63.5	8.70
38.5	18.40	64.0	8.55
39.0	18.15	64.5	8.40
39.5	17.85	65.0	8.25
40.0	17.60	65.5	8.10
40.5	17.40	66.0	8.00
41.0	17.15	66.5	7.85
41.5	16.90	67.0	7.70
42.0	16.70	67.5	7.55
42.5	16.50	68.0	7.40
43.0	16.25	68.5	7.30
43.5	16.00	69.0	7.18
44.0	15.80	69.5	7.00
44.5	15.60	70.0	6.88
45.0	15.40	70.5	6.75
45.5	15.20	71.0	6.67
46.0	14.95	71.5	6.48
46.5	14.75	72.0	6.35
47.0	14.50	72.5	6.20
47.5	14.30	73.0	6.05
48.0	14.10	73.5	5.90
48.5	13.90	74.0	5.80
49.0	13.70	74.5	5.70
49.5	13.50	75.0	5.55
50.0	13.35	75.5	5.40
50.5	13.15	76.0	5.25
51.0	12.90	76.5	5.15
51.5	12.75	77.0	5.00
52.0	12.55	77.5	4.85
52.5	12.35	78.0	4.75
53.0	12.20	78.5	4.60
53.5	12.00	79.0	4.45
54.0	11.80	79.5	4.35
54.5	11.60	80.0	4.20
55.0	11.40		

ing with the tip held above the solution in the centrifuge tube. In other words, the exhaled breath must not be "hubbled" through the dilution fluid, as the carbon dioxide of the breath may neutralize the alkalinity of the solution sufficiently to cause slight precipitation of globulin and thus cloudiness of the solution. The tube is now thoroughly shaken with a rotary motion to facilitate complete dilution. Although this minute description of this part of the technic may sound trivial, it is exceedingly important that all dilutions be made in a standard manner. Check dilutions may be made until one is sure of the technic. Usually about four dilutions can be thus prepared from one sample of blood by working quickly, as one dilution can easily be made in less than a minute.

THE METHOD OF MAKING A READING

The spectroscopic absorption cell should be thoroughly cleaned with water, alcohol, and ether. The empty container is first placed in the photo electrometer and the shutter opened. The light should be so adjusted by means of the iris diaphragm that the swing of the ammeter is exactly from 0 to 100. The shutter is then closed, the cell is filled with diluted blood and again placed in the apparatus. On opening the shutter the swing of the ammeter will be less than from 0 to 100, depending on the density of the solution of hemoglobin. As one of the oxyhemoglobin bands in the spectrum lies in the same region as that represented by the light transmitted through the filter, the increased density is indicated at once by a decrease in current flowing to the ammeter. The reading on the ammeter is readily translated into grams of hemoglobin in each 100 cc by referring either to the curve (Fig 3) or to the tabulation.

In calibrating the instrument we have made hundreds of determinations ranging from the very low readings of the severe types of anemia to the very high quantities found in polycythemia. We have also demonstrated that the time necessary for making a determination need not be more than two minutes. In fact we know of no other accurate procedure in hematology that can be performed as quickly.

In a series of experiments made to determine any fading due to standing any length of time, it was found that after two or three hours' standing there was less than 3 per cent loss in color. This fading occurs in the first two or three hours and apparently there is no further loss, as shown in readings made four to eight hours later. Readings, therefore, should be made as soon as possible after the dilution is made.

It is not in the province of this paper to discuss problems in hematology, nor to attempt to establish normal standards for the hemoglobin content of human blood. Many clinicians may find it hard to break away from an established custom of thinking of hemoglobin in terms of "per cent," based on some arbitrary figure taken as 100 per cent. In such time honored calculations as that of the so called color index, it may appear difficult to accommodate the new figure to the old method of making a ratio between hemoglobin and erythrocyte count percentage. However, if as a compromise figure for normal hemoglobin, 16.66 gm is taken as 100 per cent, and 5,000,000

is taken as 100 per cent for the erythrocyte count then the color index is easily estimated by the following simple formula

$$\frac{\text{Gm Hb in 100 cc} \times 6}{\text{RBC count in millions} \times 20} = \text{Color index, normally 1}$$

For example, if the number of grams of hemoglobin equals 15 in 100 cc and the erythrocyte count is 4,500,000 then

$$\frac{15 \times 6}{4.5 \times 20} = \frac{90}{90} = 1$$

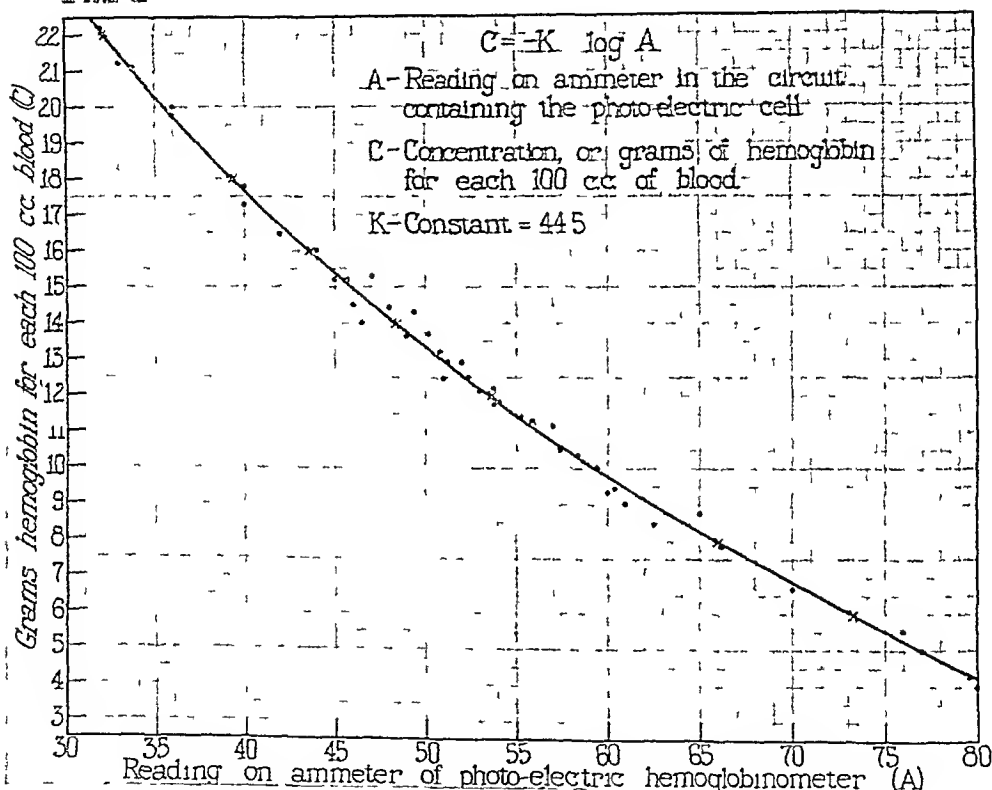


FIG 3—The relationship between the readings on the photoelectrometer and the grams of hemoglobin determined by the van Slyke method

We realize that the figure given is arbitrary and does not conform to any standard that has been established, on the other hand, it must be remembered that there is a wide variation in figures, both above and below that given, which have been reported as the normal average. It is not our purpose to discuss at this time the subject of the correct normal hemoglobin value, but merely to suggest a solution to the question as to how one may determine the color index.

In conclusion, we wish to state that we believe that the modified instrument as described and demonstrated at this meeting can be used satisfactorily for routine work, especially if a large number of determinations must be

made daily. We also call attention to the fact that the photoelectrometer may be adapted to many other uses in colorimetry. We shall prepare calibrations for the more common tests of the chemistry of the blood as soon as possible.

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DISCUSSION

Dr. Walter M. Simpson—In comparing this with the methods in ordinary use, such as the Dare, the Sahli, etc., what percentage of error is there in those methods compared with the findings with the photoelectric hemoglobinometer? How do your findings check with the van Slyke and Newcomer determinations?

Dr. A. H. Sanford (closing)—It is a very important question. Perhaps we should have gone back to last year's paper. The curves were made and checked with the van Slyke oxygen capacity method. That is considered the standard method. Frankly, I believe this photoelectric instrument is a better method so far as accuracy is concerned than the Newcomer method and checks within 1 per cent. Several hundred colorimetric determinations one right after the other are very tiring to the eyes. I know that this photoelectric method will relieve the technician a great deal in routine work just in that one respect of relieving eye strain. Technicians also differ in the way they match colors. We hope the photoelectric eye will stand up and not get fatigued.

RHAMY TRIPLE STAIN FOR FROZEN SECTIONS*

By B W RHAMY, M D, Ft WAYNE, INDIANA

FROZEN section tissue diagnosis in the operating room has become so deservedly popular that improvement in the technic should meet with favor. In my hands the greatest drawback was a satisfactory stain. Unna's polychrome methylene blue worked if one was fortunate enough to succeed in making a good batch, but it always seemed that when the time came for using it, it was deteriorated. Besides a permanent mount could not be made. Terry has improved this stain wonderfully but his stain also has the drawbacks, that it is difficult to make, fades quickly, cannot be used on all tissues, and tissue stained with it cannot be mounted permanently. Turley has suggested a quick hematoxylin-eosin stain, which however has many steps, and, in my hands failed to produce satisfactory stains in the time given, due to the fact that unfixed tissues do not take stain as readily as do fixed tissues. Of course frozen section tissue diagnosis must be done quickly, the staining method must differentiate the cells and their arrangement in the tissue so that their pathology may be quickly recognized.

I offer the following staining method as one that I believe fulfills these essentials and having the additional advantage that the section examined can be mounted permanently and that it does not fade.

STOCK SOLUTIONS

Solution I—Saturated alcoholic solution basic fuchsin for bacilli (Grubler)

Solution II—Saturated aqueous solution eosin

Solution III—Saturated alcoholic solution methylene blue for bacteria

Solution IV—Thirty per cent grain alcohol

PREPARATION OF STAIN

Take of Solution I	3 to 5 c c
Solution II	5 c c
Solution III	15 c c
Solution IV	q s to 100 c c

The color of this mixture should be purplish blue. The fresh stain can be used at once but is better after ripening for forty-eight hours. Should the color be blue or reddish blue, due to atmospheric conditions or to poor quality of dry stains, let stand to ripen for a week or two. When stain is right, *B. coli* stains violet while *B. typhosus* and *B. paratyphosus* stain pink, *B. diphtheriae* rods and bars stain blue with pink interspaces.

*Demonstrated before the American Society of Clinical Pathologists Portland Oregon July 5, 6 and 8 1929

METHODS FOR FROZEN SECTIONS

1 *Wet method* Cut tissue into 4 per cent formalin Float section on slide, cover with stain, and count 40 Rinse with water or dextrose solution and examine

2 *Permanent mount (time sixty seconds)*

- 1 Cut tissue into 4 per cent formalin solution
- 2 Section can be floated on slide and stained or can be lifted with a needle or bent glass rod and dipped in the solutions
- 3 Cover with stain ten or fifteen seconds (count 30 or 40)
- 4 Wash off excess stain with water
- 5 Ninety six per cent alcohol (or denatured) until excess stain is removed (five to ten seconds)
- 6 Absolute alcohol three to five seconds
- 7 Xylol three to five seconds
- 8 Mount in neutral xylol balsam

3 *For Terry's razor sections* This stain can be substituted for Terry's stain The stained picture is practically the same by both stains as shown by simultaneous staining on the same tissue by Dr Terry and myself at the meeting of the American Society of Clinical Pathologists, Portland, Oregon July 5 to 8, 1929 The difference between the stains was that my stain takes about five seconds longer to produce the same depth of stain The simplicity of preparation of my stain its keeping qualities, and the fact that permanent mounts can be made with it are its advantages

QUICK METHOD FOR MOUNTED SECTIONS (TWENTY FOUR HOURS)

- 1 Five to 10 per cent formalin one hour
- 2 Acetone two changes one half to two hours each depending on size of the block of tissue
- 3 Acetone, absolute alcohol ether, equal parts one half to two hours
- 4 Absolute alcohol and ether, equal parts one half to two hours
- 5 Thin celloidon one half to two hours
- 6 Thick celloidon, overnight
- 7 Mount and cut
- 8 Lift section with needle and immerse in stain (count 30)
- 9 Wash in water
- 10 Ninety six per cent alcohol (or denatured) to differentiate or until stain ceases to come out (about ten seconds)
- 11 Absolute alcohol
- 12 Xylol
- 13 Mount in neutral xylol balsam

This stain can be used on any sections of hardened tissue whether frozen, mounted in celloidon or paraffin It is an excellent counter stain for tubercle bacilli in tissues

STAINING PROPERTIES

This stain tends to differentiate itself after mounting. Sections can be left in alcohol a reasonable length of time but can be decolorized if left unduly long or by prolonged exposure to direct sunlight.

Epithelial cells take a light blue stain, endothelial cells lavender and small round cells deep blue. Glandular epithelial cells take on a sky blue, the nuclei of spindle cells stain a lavender blue. Connective tissue fibers stain pink while muscle fiber takes rose red to magenta. Mast cells show dark blue with dense blue granules. Erythrocytes stain pink to orange, lymphocytes deep blue with magenta cytoplasm. Polynuclear cells show blue nuclei with pink cytoplasm. Concretions and mucus stain blue while caseous material stains pale red. Degenerated areas stain light blue to pink. Spermatozoa stain with the head blue, connecting piece dark red and tail pink.

While this stain is intended for tissue it is useful as a triple stain for blood smears and for bacteria.

For Blood Smears (Quick Stain)

Better than eosin-hematoxylin and about as good as Wright's stain.

1. Fix blood smear with denatured or other alcohol.
2. Wash or blot.
3. Cover with stain one minute.
4. Wash with tap water, blot and dry.

Nuclei blue, neutrophile cytoplasm pink. Lymphocyte cytoplasm red to reddish blue. Basophiles same as in Wright stain. Eosinophile granules pink to opaque white (like crystals). Red cells pink to orange, polychromatophilic cells red.

As a Stain for Bacteria in Smears and Cultures

1. Fix with heat.
2. Cover with stain one half to one minute.
3. Wash and dry.

Negri bodies stain magenta with blue granules, nerve cells light blue. B. typhosus and B. paratyphosus A and B stain pink. B. coli and B. dysenteriae stain light to dark lilac. B. diphtheriae beads and bars stain blue and interspaces pink. B. influenza and leptothrix, bars and polar bodies lilac, interspaces colorless. Pneumococci blue with capsules in sputum glistening white in a red field. Staphylococci and streptococci blue (some streptococci stain pink). Gonococci deep blue (stand out in vaginal smears). Meningococci (in cultures) live cocci lavender, old cocci pink. Mic. catarrhalis (in cultures) live cocci blue, old cocci pink. Blastomyces (yeasts) blue, capsules pink. Spirochetes some pink, some lavender. Mould mycelium dark red or lavender, spores blue, capsules colorless. Spores white, beads with violet capsules.

I have tissue, blood and bacterial specimen slides which were stained by these methods in 1927 which are still well stained. Some of these slides were shown at Portland last year. In making this stain be sure to have reliable dyes. My experience with some makes was disappointing.

THE EFFECT OF BILE ON THE AGGLUTINATION REACTION*

By RUTH GILBERT, A M M D, AND MARION B COLEMAN B S,
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OF THE factors that may lead to irregularities in agglutination tests for typhoid and paratyphoid fever variations in the cultures used are probably the most important Besides the differences in agglutinability of various strains of typhoid and paratyphoid bacilli cultures maintained in broth or on agar, which have been satisfactory for weeks or months, may suddenly show spontaneous clumping, loss of motility or other undesirable qualities The bacteriolytic property of certain sera, if not rendered inactive may also furnish a source of error In a study of means whereby this property might be inhibited, it was found that heating at 55° C for one half hour¹ while otherwise satisfactory at times lowered the agglutination titer Sodium citrate likewise proved effective but a concentration sufficient to inactivate completely the lytic properties is hypertonic Ritti² has reported that this reagent eliminates the so called zone of inhibition in sera Of the various methods studied the use of bile in the culture medium or as a diluent of the sera proved the most satisfactory, since it was found not only to inactivate the lytic properties of the serum, but also to have a favorable action on cultures grown in medium containing it

It is generally known that the growth of *B typhosus* may be stimulated by the presence of bile in culture media especially in those used for isolating the organisms from blood Hauduroy³ Caublot⁴ and De Jong and Hauduroy attribute this to a destruction of the lytic activity of the bacteriophage Our results can hardly be explained by this theory however, since the presence of bile has inhibited the lysis of killed as well as living organisms Pfannenstiel⁵ and Pfannenstiel and Kortman⁷ have shown that the presence of bile or bile salts renders inactive the bactericidal, bacteriolytic and other complementary properties of sera Formicola⁸ reports that bile accelerates the agglutination reaction and may increase the titer of serum markedly

During two years, our staff members have performed in duplicate 350 microscopic tests using typhoid bacilli grown for eighteen hours at 37° C in extract broth and in peptone water medium containing 3 per cent fresh ox bile, the optimum amount indicated by preliminary tests The stock cultures were kept on infusion agar

Preparation of Bile Medium—Combine 1 kg distilled water 10 gm peptone, and 30 gm fresh (5 gm dehydrated)* ox bile and boil vigorously for from three to five minutes Leave in a cold room overnight and filter

*Read before the Eighth Annual Convention of the American Society of Clinical Pathologists Portland Oregon July 5 6 and 8 1929

From the Division of Laboratories and Research New York State Department of Health Albany New York

Peptone water containing 0.5 per cent dehydrated bile has been used with equally good results providing it is freshly filtered A precipitate forms more rapidly in this medium than in that containing fresh bile

through paper until clear Dispense in bulk and sterilize in steam at 121° C for twenty minutes Store in the cold room Decant or filter through paper before tubing and sterilize in steam at 121° C for twenty minutes Since solutions containing bile develop precipitates, medium containing it should be used within a week after filtration

The specimens examined included dried blood and sera The tests were made by the hanging-drop method and incubated one hour at 37° C Usually the work was done by two individuals, and the results were compared after completion No lytic activity was observed with the culture in medium containing bile, while lysis was frequently so marked with the one in broth that it was necessary to repeat the examination after inactivation of the serum Otherwise, appreciable differences did not occur at any time in the reaction obtained with the two cultures The usual fluctuation in motility and the variation in the character of the clumping in the broth culture were noted from time to time, but the culture in media containing bile was uniform

Cultures grown in bile medium have not been found entirely satisfactory for macroscopic agglutination tests, the lytic properties of some sera remaining active when tested with this culture However, excellent results are obtained with sera diluted with 0.85 per cent salt solution containing 3 per cent fresh ox bile and allowed to stand for a short time before the addition of either a living broth culture or a formalinized suspension in 0.85 per cent salt solution

SUMMARY AND CONCLUSIONS

When cultures of *B. typhosus* or *B. paratyphosus* have been grown in media containing appropriate amounts of bile, no evidence of loss of motility, spontaneous agglutination, or lysis has been noted in microscopic agglutination tests Also, the addition of similar amounts of bile to the salt solution used in diluting sera for the macroscopic agglutination test has rendered their lytic properties inactive The action of the bile may be due to a lowering of the surface tension, but further study is needed to determine the basis for the reaction

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GINGIVITIS*

V THE CHARACTER OF THE EXUDATE IN GINGIVITIS

By ROBERT A. KEILTY M.D., WASHINGTON D. C.

THIS is the fifth in a series of papers on the general subject of gingivitis, and it is presented from the bacteriologic viewpoint. My approach to the subject will be found in previous reports^{1 2 3 4}. While the problem as a whole rightfully and strictly belongs to the dentist, this phase of the report may interest those working in mouth bacteriology.

As one studies the bacteria of the mouth the maze of possibilities becomes endless. It is far from the simple isolation of a streptococcus, a staphylococcus, or a diphtheria bacillus as outlined in the textbooks. Where one delves into the relationship of the bacteria found in thousands of months and attempts to check the findings against the pictures of gross pathologic conditions, as viewed from the conceptions of the pathologist, the problem at times seems hopeless.

By gingivitis I mean any inflammatory process involving the soft tissues about the necks of teeth. Where the lesion has extended to the bone the term gingivitis may be augmented by periodontitis. As far as the exudate is concerned all factors are included under gingivitis. I am not attempting to discuss causal relationship here, and I am mindful of all the other factors which may be present in a given case. These have been discussed in other papers. It would seem wise to group all forms of inflammatory changes in the gums from acute trench mouth and Vincent's angina to pyorrhea under the general terms of gingivitis and stomatitis qualified by their appropriate descriptive term. This is especially true the more one studies the bacteriology and tends to simplify the subject as a whole right in the beginning.

In the first place while the critical study of a thousand or five thousand mouths will place them in groups subject to classification, after all, each individual mouth is an entity and the factors found must be evaluated per se as far as that particular mouth is concerned. Great individual variation occurs.

The perfectly normal mouth is a rare condition. The gingivae are involved to some extent in over 90 per cent of hospital admissions and even higher in general dental practice. Normal gums⁵ are firm and pale, abnormal gums are red and inflammatory in appearance. Normal gingival sulci are tight and contain nothing but a few desquamated cells, an occasional leucocyte and very few bacteria, if any, on smear. The presence of actual material about the necks of teeth and in the gingival sulci constitutes an exudate. Many mouths, where cleansing is not a routine after eating, contain foodstuffs about the necks of teeth but this is easily differentiated from exudate. The exudate is, of course, the product of reaction from the subgingival and periodontal

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time might be discarded. At the same time the importance of maintaining the necessity for a symbiotic relationship with fusiform bacilli could be discarded. Let us consider *B. fusiformis* and spirilla as definite entities until they are more satisfactorily studied. As far as the spirochete organisms are concerned we are now at the stage the streptococcus was in several years ago when it was spoken of as *S. longior*, *S. brevior*. These were mere terms and meant that then terminology represented the state of our knowledge at that time.

Borrelia vincenti appear constantly in gingival exudates. They are best seen in fresh saline smears under the high dry powers of the microscope. As a rule the more active the infection is the longer the forms are, the more active they are and the more spirochetes they have as compared with the looser more undulating types seen in chronic cases. In these acute cases at times they closely resemble *T. pallidum* under the dark-field and while they can as a rule be differentiated, occasionally this is impossible. For those who are not perfectly familiar with *T. pallidum* under the dark-field a positive diagnosis in acute suspected syphilitic lesions of the mouth should be fairly certainly made before one is too dogmatic on the differentiation.

Considering all spirilla not definitely *Treponema pallidum* found in the mouth under the generic heading of *Borrelia vincenti*, they are subject to description. Examined in fresh saline suspension, they vary from 3 to 15 microns in length and are uniformly thin with slightly pointed ends. Usually approximately the same size holds for the predominant type present in a given mouth. As a rule the more chronic the case is the shorter the prevailing forms are and the more acute the case the longer the forms are. In the same way the more inactive the process is the more sluggish the organisms are in movement and the more acute the case the more active. In activity they have a characteristic movement which is sharp and jerky with an instant period of complete quiet. They may move in a very small sphere backward and forward, or they dart in all directions. They are gram-negative as a group, and when gram-positive forms are seen, something is usually wrong with the stain. They take several stains including fuchsin and gentian violet. In stained preparations they are more drawn out with an undulating wave and are, therefore, correspondingly longer. I have seen at times a wavy membrane but this is not the rule. At times in the dark-field they seem to have either a splitting longitudinally or actual flagella but this also is not the rule. Their bodies are uniformly stained and intracellular granules is not the rule.

Many attempts at cultivation have been made but nobody has described entirely satisfactorily a method which will allow definite pure culture studies. I have tried many possibilities. At times anaerobically I have thought I had more than a transfer but they always die out. Recently I have had some actual growth on twenty-four-hour blood agar plates in apparent symbiosis with staphylococcal colonies. I have thought at times that fusiform bacilli were showing evidence of transmutation to spirilla but I have never had any actual success. I know of several studies now being carried out along anaerobic lines for the cultivation of spirilla and any one of these may prove

successful any day Unless spirilla can be made to start under aerobic conditions they must be started in mixed culture first and eventually, by transfer, made to grow at the expense of their symbiotic fellows and not otherwise as now happens

In the gingival exudates, spirilla classed under the genus *Borrelia* can be easily detected by fresh, wet smear or by stained smear and their presence indicates inflammatory action They are not present in normal mouths and they disappear in cases appropriately treated They are transferred, of course, from mouth to mouth mainly by kissing At the present time there is a marked increase in cases of acute ulcerative gingivo stomatitis (trench mouth), due in part to great numbers of active borrelia which probably represent virulent strains of the organism

The next most common organism in gingival exudates is the fusiform bacillus This is also loosely described in textbooks but in *Determinative Bacteriology*² is under Order II Actinomycetaceae Family II, Myobacteriaceae, Genus III, Fusiformis, No 2 Fusiformis dentium, Hoelling It is ten to twelve microns long, slightly curved with pointed ends It is usually not motile but at times it is quite motile, swimming about in a slow graceful fashion It is gram negative While it may be obtained both on blood agar plates and in anaerobic cultures or by a combination of both it is difficult to obtain in pure culture It is readily recognized in wet and stained smears, varies in numbers and while it is usually present in the same smears with borrelia it may appear independently It is not present in perfectly normal mouths

The next organism in order of frequency is a small comma like bacterium more like a tiny polywog with a small body and a tail, not an actual flagellum This has the greatest motility of any of the motile forms and darts all over the field It is apparently described under a variety of names and again until more definitely placed, *Vibrio sputigenus* (M D B³) best places it

There is a more or less constant longer broader bacillary body with blunt or even square ends, nonmotile sometimes in pairs or short chains and always gram positive This belongs to the lactobacillus group It has recently been described as an associated cause of dental caries but it is present in most exudates without any evidence of caries

Under the more or less general term of leptothrix, mycelia are present in most exudates but more in the sordes of neglected mouths and in lessened numbers in the more acute and advanced cases of gingivitis These appear as long interwoven mycelial threads, are nonmotile and as a rule gram positive Occasionally branching is seen but this is not the rule From cultures in symbiosis with the coecal colonies they appear as long swollen threads with many intracellular bodies They are the least pathogenic of all the various types since they appear in abundance in the least active mouths

In many exudates there are found clumps of bacilli of the gracefully moving fusiform type, which are in three formations First they are occasionally definitely agglutinated, much more often they appear as developing rosettes There is a central body mass and from the periphery the bacillary bodies protrude as in a ray fungus In the third form the bacillary bodies

are directed and folded in one direction so that the appearance is that of the tuft of a pineapple. Similar rosettes were shown to me by Broders last year in some tonsil sections. I am not sure where they belong but they are a frequent finding in gingival exudates.

There are occasionally other forms of fungi, mycelia and yeasts. They are inhabitants of individual mouths and do not belong to any group constantly found in exudates. The yeasts which have been suspected by some writers are not present in numbers of cases but occasionally a single case will show them in abundance.

The other bacteria present in exudates can best be determined by cultural methods and the blood agar plate brings out most of them. The flora is more or less constant for a given mouth, shows the same type from individual sulci and pockets with variations of predominant colonies from different locations. The exudates are influenced by seasonal changes, heavier in winter and lighter in summer. For example the pneumococcus, predominant in winter, almost disappears during the summer. Gingival floras bloom in types at epidemic times during the winter and keep pace with the general tonsil and pharyngeal floras. There are forty six different varieties of bacteria described in the *Manual of Determinative Bacteriology* as having been isolated from the mucous membrane of the mouth or from the sputum. I believe I have isolated thirty-three of these different types from gingival exudates in this work so far and I know this will increase as the cultural studies are augmented and elaborated.

Different forms of staphylococcus and streptococcus are present in all exudates. The characteristic staphylococcus is a muddy, moist, glistening, mushroom, jelly-like colony in which other forms of white cocci grow and in which fusiform, bacillary, mycelial and vibrio symbiosis appear in the colony. This is especially true in the heavy parts of the smear and at points where it is confluent. The pure white and yellow staphylococcus is not the rule but at times predominates. At other times deep yellow and pink colonies appear in the older plates. The muddy staphylococcus is usually hemolytic, at times markedly so and at other times shows an opaque sheen in the zone of the hemolysis. The dense muddy colonies with gram-negative cocci are those of *Neisseria catarrhalis* (*Micrococcus catarrhalis*). The staphylococcus and the catarrhalis are closely allied and often only in the gram stain can they be differentiated, those cocci which are positive being staphylococci and those negative catarrhalis. Many of these colonies with gross variations distinguishable only under the dissecting microscope show marked variations in the cocci when stained. They appear as globoid bodies often with variations in size, masses, tetrads, diplococci and continue to transplant in their form or change markedly from plate to plate. There are characteristic muddy colonies of the general type of staphylococcus in all exudates which represent different strains in different mouths.

The streptococcus is subject to an even wider variation. The colonies are flat, moist, gray, pearly white, opaque and digesting. The hemolysis is small, marked, sharply defined, and green. The virid hemolysis predominates in most of the cultures. Streptococcal groups may be designated by their blood agar plate reaction and I am now working on their serologic grouping.

There are many other organisms isolated from the exudates of particular cases. In a recent unselected group of one hundred cases¹ showing all types of gingivitis, in addition to the different strains of the streptococci and staphylococci groups which make up the vast preponderance the following organisms were found pneumococcus, B diphtheria B hoffmanni yeast and an unidentified bacillus. At times I have found B coli B influenza but never B typhosus nor B tuberculosis.

The protozoa in the mouth form a very important link in the etiology of gingivitis. *The generally accepted viewpoint that they are harmless parasites is erroneous.* They apparently do not produce metastatic lesions outside of the gingivae but in the gingival sulcus they are most active irritants in the continuation of suppurative processes. It is not my purpose to review the literature nor the controversy here but merely to state their findings.

The most frequently occurring protozoa is *Endameba gingivalis* (Gros). This organism is found in 71 per cent of my cases. It appears most frequently in the deep pockets of the more chronic cases but it also may be found in acute cases. It is present in association with the other flora. It is not by any means the only etiologic factor; it is not a specific cause of pyorrhea but it is an important factor. It may in a very small group, less than one half of 1 per cent, be the one factor which will clear the case up and in those cases it is of prime importance.

*Endameba gingivalis*¹ has been well described by Smith Bass and Johns Kofoid and Swezy and others. I would like to add here and present evidence later, that in addition to the vegetative forms it occurs in cyst form in common with other endameba. I have no evidence yet that it invades the tissue of the gingivae or that it appears in the intestinal flora in these cases.

The next most common protozoa is the trichomonas. This was first described by Steinberg 1862 who named three distinct forms (Wenyon). Wenyon suggests the generic name *Trichomonas elongata* the first proposed by Steinberg as against *T buccalis* of Goodey and Wellings. On the other hand in order to differentiate easily from *T vaginalis* and *T hominis* *T buccalis* (Steinberg) would seem appropriate. Lynch² has studied the organism extensively in cultures. I have found it several times very abundantly in an aerobic culture, sometimes it is found after a week's incubation when it was not seen in the original smear. In a series of positive gingival cases in which the feces were studied *T hominis*, identical with *T buccalis* was found in two cases. I believe after more careful search in a greater number of cases this protozoa will be found more frequently in both the mouth and feces and the relationship between the two more definitely decided. *Trichomonas* in the mouth is as a rule much smaller than *T vaginalis* and more nearly the size of *T hominis* in the intestine. In my estimation this parasite occupies the same relative position as to pathogenicity in gingivitis as does the endameba.

There is another much smaller protozoa which I have frequently seen and only lately have been more carefully searching for. This is a small motile cell with either a single flagellum as in the species *Bodo* or it is a form of infusoria with an attachment process. This is of frequent occurrence and warrants more critical study.

I am sure I have seen one or two definite *Giardia* in gingival exudates. For the past year I have been closely on the lookout but have not encountered a case. It is evidently very rare. The same may be said of *Cercomonas*. In my early work I have mistaken *Cercomonas* for *Trichomonas*, since I have not seen a single instance of *Cercomonas* during the past year. There are no other protozoa, neither vegetative nor cysts with which I am familiar, that have been seen in the gingival exudates thus far studied. I have been looking for *Blastocystis hominis* but do not believe that it occurs in the mouth.

DISCUSSION

There is a general tendency, because of the conditions present and the consideration of bacteria and protozoa as inhabitants of normal mouths to disregard their importance in pathologic lesions and to look for other factors in etiology. The more one studies these bacteria the more important they become and with treatment directed at them the greater is the improvement in results. The exudate about the sulci of teeth is even more complicated than the fecal flora, but it can be worked out relatively satisfactorily so that individual cases may be treated more intelligently.

Since the pathologic changes in the gingivae and bone about the teeth of an inflammatory character have all the earmarks of an infectious nature, it seems reasonable to give the bacteria and protozoa found their rightful importance and consider them in a direct etiologic rôle.

The apparently widely separated conditions known as "trench mouth" and "pyorrhea" become closely related on the basis of the study of their etiologic organisms. For this reason I have grouped all inflammatory changes in the gingivae under the heading of gingivitis and have worked out for each case its etiologic flora.

In studying the exudate, its gross and microscopic appearance must be considered from a purely cellular exudative standpoint. In the mouth ex-taneous cytology introduced in foodstuffs must be differentiated. The protozoa, *Endameba* and *Trichomonas*, are more or less common with *Cercomonas*, *Giardia*, *Balantidium* and *Bodo* occasionally seen. The spirilla under the name of *Borrelia vincenti*, the fusiform bacillus, *B. acidophilus*, *Vibrio sputigenus* and mycelia are constant findings. The staphylococcus and streptococcus groups are also constantly present. A large number of other organisms may appear in individual mouths such as the pneumococcus, *B. coli*, *B. influenza* and virulent types of *B. diphtheriae*. All of these organisms have a direct bearing as etiologic factors of the gingivitis per se. In addition, the invasion of the subgingival tissue acting as a portal of entry becomes one of the most important focal points of infection for the production of metastatic pathology.

CONCLUSIONS

Definite gingival exudates appear in the sulci about the necks of teeth which are subject to a bacteriologic study in relationship to the disease present in the gingivae.

Perfectly normal mouths do not have exudate about the necks of teeth, therefore, the presence of exudate about the necks of teeth is an indication of inflammatory change in the gingivae.

On the basis of the bacteriologic study of gingival exudates such diseases now known as trench mouth, Vincent's angina and pyorrhea become different manifestations of a single entity, gingivitis.

The prevailing organisms are *Borrelia*, *B. fusiformis*, *Vibrio* *sputigenus* a bacterial flora *B. acidophilus* and *myceba*.

The prevailing protozoa are *Endameba* and *Trichomonas*.

The study of the bacteriology and cytology of gingival exudates places a nonspecific bacterial etiology for gingivitis of equal if not of greater importance than the other factors now considered predominant by the dental profession.

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- 6 Wenon, C. M. *Protozoology*, Vol. 1, 1926. Wm. Wood & Co.
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DISCUSSION

Dr. M. M. Patton.—This question of mouth bacteria is an important one and something which needs further work both in the cultivation and isolation of the bacteria causing it. It is surprising to find in some mouths where they have large ulcers etc. that you will find very few spirilla. In other cases where they have small ulcers or single ulcers it is surprising how many more spirilla you will find. I was glad to hear Dr. Kelly say that at the present time he paid no attention to the number of bacteria present. I think it is rather important that he does not try to classify the bacteria. We find in mouths where there are a great number of bacteria and where they are extremely motile that these are the worst cases. The dentists in our town are cognizant of the fact that removing teeth promiscuously without first having smears taken from the teeth is not a good procedure. I have seen large ulcers and death from just the removal of teeth. Some men think that the spirilla of the mouth have something to do with certain forms of anemia particularly infectious mononucleosis and allied types. It occurred to me that there might be some relationship of the bacteria of the mouth particularly the spirilla to chronic myocarditis and arteriosclerosis. In the work of Warthin, in which he stained the tissue of the heart in sclerosis of the heart muscle, he almost always found spirilla and classified them as *Treponema pallidum*. This view has not been entirely accepted by others but it might be well to think of these treponemes as some other form of spirilla closely resembling pallida types.

Dr. Frederick H. Lamb.—I have wondered if others have thought as I have about expressing the volume of the infected area in figures. If the diameter of the average tooth is about one fourth of an inch, the circumference of the tooth is about three fourths of an inch and if the depth of the infection is three eighths to one half inch it is easy to figure that, if there are twenty infected teeth the infected area is equal to a ribbon of infection approximately fifteen inches long and from three eighths to one half inch in width. One need have no doubt then about the teeth being a prolific source of infection since we have an enormous number of bacteria in potential contact with the blood stream.

In regard to the last speaker's reference to Dr. Warthin's work on spirochetes I

can say with certainty that the organism which Dr Warthin speaks of as being *troponema pallidum* is without any doubt that organism

Dr E C Rosenow—The facts brought out by Dr Keilty are unquestionably of the greatest importance. Infections of the gingivae as a source of general ill health and the source of grave systemic disease has scarcely been considered by physicians and dentists. His presentation leaves no doubt that gingivitis is an important source of such infections. Of the numerous kinds of microorganisms at hand, none is perhaps as significant as the streptococcus, at least as far as relation to systemic disease is concerned. Drs Cook and Stafney working with me in the section on Dental Surgery at The Mayo Clinic have shown conclusively that the exudates in periodontitis contain streptococci of the viridans group like those isolated from the apices of pulpless teeth and which also tend to localize and produce lesions in animals similar in location to those at hand in the patient from whom the material is obtained, elective localization.

I hope that Dr Keilty's presentation will do very much to awaken interest in this important and greatly neglected source of infection.

Dr Frank W Hartman—I would like to ask if Dr Keilty sees any constitutional disturbances in any of these cases, especially in the marked cases.

Dr Robert A Keilty (closing)—The point that Dr Hartman has just raised, might be discussed by some of the California men. A group in California working on etiologic factors dropped off it seems to me just as their evidence was convincing. They gave infectious factors a secondary rôle and constitutional disturbances a primary rôle. As a primary rôle a constitutional disturbance plays an important but a minor part in this group of cases. Tuberculosis and diabetes are secondary to other more definite gingival factors.

In answer to Dr Rosenow, I am glad to know that Dr Cook has shown that these organisms do the same as those isolated from the deeper pathologic areas. In fact the avenue of infection or portal of entry is more commonly by the gingival route than it is through the root canals in my opinion. There is no reason whatever for extracting a tooth because it is just dead, especially when it does not show any periapical pathology. Many dead teeth with root canal fillings are sterile and will remain so for a number of years.

The last point, there are entirely too many deaths starting in the extraction of a single tooth and ending in cellulitis of the face and neck. These are preventable cases when a mouth has had preoperative specific treatment from the standpoint of the gingival infections present. Under these conditions any amount of surgery can be done at one time when the danger of secondary flare ups is reduced almost to nothing.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE M.D. ABSTRACT EDITOR

C DIPHTHERIAE Hydrolyzed Serum Agar for the Isolation of *C Diphtheriae* Thompson L. J Infect Dis 45 163, 1929

A serum agar has been prepared by treating Loeffler's blood serum, dehydrated with sodium hydroxide and incorporating the nonconglutinated product in agar. The medium promotes the growth of diphtheria organisms, and inhibits certain other organisms found in the throat. It is easily available, easily prepared, can be safely sterilized in the autoclave and offers the convenience of an agar medium for the plating and isolation of pure cultures of *Corynebacterium diphtheriae*.

The formula follows:

Loeffler's blood serum (Bacto dehydrated), 40 gm, water (at 40° C), 250 cc mix well, sodium hydroxide (normal) 150 cc, place in incubator at 37° C for forty-eight hours, neutralize with 5 per cent hydrochloric acid to P_H 7.0 using bromthymol blue, add 25 gm sodium citrate, when dissolved, titrate to P_H 6.4 with 3 per cent citric acid, to this add, while hot, an equal amount of 3 per cent agar solution. Put in tubes or flasks as needed and sterilize in the autoclave at 15 pounds pressure for fifteen minutes.

VEREUGA Cultivation of *Bartonella Bacilliformis* Battistini T S Ann Facul de Med Lima 28 No 10, 243, 1927

The author's technique for cultivating *Bartonella* is quite simple. A small drop of blood from the finger of the patient is withdrawn by means of a pipette into semisolid medium (serum agar, for example), the end is sealed in the flame and the whole placed in an incubator at 28° C. Colonies are visible in five to six days. The individual bodies are 0.6 x 0.2 microns to 1.6 x 0.5 microns. They are gram negative, markedly motile at the junction of medium and water of condensation, but flagella have not been demonstrated. In old cultures spirillar forms are seen, from 7 to 30 microns in length. Growth will not take place in fluid media, only on solid or semisolid and under aerobic conditions. Cultures had no action on any of the 17 sugars tested. The reaction of the medium may be between P_H 6.8 and 8.2, but the optimum is 7.4 to 7.6. A temperature of 56° C destroys the organisms in ten minutes of 60° C in five minutes. 1 per cent formalin, tricresol, or lysol in ten minutes.

Whether in culture or blood they will not pass through Berkefeld filters V or N. In citrated blood they remain viable at 14° and 26° C for three and one month respectively, in culture at laboratory temperature (22° to 25° C) they survive for sixty days even though no precaution is taken to prevent the medium drying.

STAIN A Modification of Mayer's Hemalum Sasser J E Stain Tech 4 127 1929

Dissolve 50 gm of alum $Al_2(NH_4)_4SO_4$ in a liter of boiling water. Remove from the hot plate and add 1 gm of hematoxylin (obtained from Coleman & Bell National Aniline Co., or MacAndrews and Forbes). Add 1 gm $NaIO_3$ cool and filter. The stain should be filtered whenever a metallic 'scum' is present. The solution is best when fresh, but its staining properties are retained for at least six months. The keeping qualities are now being tested.

The slide to be stained is transferred from water to the staining fluid. It is washed in distilled water then in tap water (or 1/100 Na_2CO_3), and again in distilled water. Finally it is dehydrated and cleared as usual. An aqueous or alcoholic counterstain may be used. This is primarily an histologic stain.

MOSQUITO An Improved Method of Mounting Mosquito Larvae, Gater, B A R Bull Entomol Research 19 367, 1929

The mounting medium used consists of (in percentage) water, 100, picked gum arabic, 80, chloral hydrate, 740, glucose syrup 50, glacial acetic acid, 30

The living larva is placed on a slide and all surplus water is removed, some of the medium is dropped on it, and then a cover glass. Clearing begins as soon as the larva is dead (and with most species is complete in two hours, but with highly pigmented species not until perhaps twenty four hours). After mounting for permanence the preparations are set aside to dry, a process that in a moist atmosphere may take three weeks, before being ringed. "The advantages claimed for this method are simplicity of technique and efficiency of clearing."

DIPHTHERIAE A New Suspending Medium for Intracutaneous Virulence Test, Stone, R B, and Weigel, C Am J Pub Health 19 1133, 1929

STONE WEIGEL VIRULANCE TEST SUSPENDING MEDIUM (AGAR PEPTONE BROTH)

Oven dried agar	0.2%	0.2 gm
Water to make		100 cc
Boil briskly to dissolve agar, then add		
Peptone (Witte's)	4.0%	4.0 gm
Sodium chloride (c p)	0.5%	0.5 gm
Beef extract (Difco)	0.3%	0.3 gm
Dextrose (Difco)	0.2%	0.2 gm
Boil at this stage in double cooker		
Adjust to pH 7.0		
Then add n/1 Na(OH)	0.7%	0.7 cc

Sterilize at 15 pounds pressure for thirty minutes, after tubing, 1 cc per tube. Cork tubes and seal with paraffine. Care must be taken to prevent evaporation of stored medium since a very slight concentration increases the percentage of agar sufficiently to solidify the fluid. Different lots of shredded agar vary in "setting" properties, so each new lot should be tested.

UNDULANT FEVER The Diagnosis of Br Abortus Infection in the Udder of the Cow, Torrey, J P Am J Pub Health 19 360, 1929

By the application of the Huddleson rapid agglutination test to the milk, practically all animals carrying Br abortus in the udder can be detected. The first few streams from each quarter are drawn into clean test tubes or vials. The strippings should never be used. A composite sample is of little value since only one quarter may be infected. About 3 drops of rennet are then added to the milk and mixed thoroughly. The tube may be put either in an incubator at 37° C or in warm water at about 40° C. If the tubes can be placed in a slanting position, the curd will settle to one side and clear milk serum, with which the test is made, will separate. Serum will form in about 2 hours and may then be used the same as blood serum in the rapid test described by Huddleson. Tests may be made as soon as the serum separates, but, if the sample can be placed in an ice box or cold place, the fine particles of curd will settle and leave a much clearer serum. Sour milk will give an unreliable test. Milk in which the curd has become partially digested cannot be used as this interferes with the test.

FLAGELLA Stain for, Farconi, A Bull d'histol appliq à la physiol 6 306, 1929

The writer describes the method for staining bacterial flagella proposed by Petragnam in 1922. The first step is mordanting of the carefully prepared bacterial films in a mixture of the following solutions

Solution A

Tannic acid (purest)	70 gm
Forrie chlorid	350 gm
Ethyl alcohol	350 cc
Distilled water	150 cc

Solution B

Potassium alum (crystals)	30 gm
Zinc acetate (crystals)	05 gm
Acetic acid, glacial	30 drops
Distilled water	100 cc

After washing the preparation is stained cold in Ziehl's carbol-fuchsin in geatin violet, or in a saturated alcoholic solution of crystal violet in aniline water (Formulas not given)

STAIN Combined Nuclear and Differential Brimyer G J Science 68 114 1928

- 1 Stain sections in Delafield's hematoxylin for five minutes
- 2 Pass through distilled water to remove excess stain
- 3 Stain in 0.2 per cent aqueous solution of acid fuchsin for one minute
- 4 Pass through distilled water to remove excess stain
- 5 Stain in the following solution for two to three hours

Anilin blue (water soluble)	05 gm
Orange G	20 gm
Phosphomolybdic acid (1 per cent aqueous solution)	1000 cc
- 6 Pass through distilled water to remove excess stain
- 7 Pass successively (rapidly) through the following grades of alcohol 30 per cent 70 per cent and 95 per cent
- 8 Complete dehydration in absolute alcohol in one half to one minute (Water free acetone may be used in place of absolute alcohol with but little or no shrinkage of the cells)
- 9 Clear in xylol
- 10 Mount with cover glass

With this staining nuclei will appear a rich red epithelial cells pink connective tissue blue and muscle red. Red blood cells will stain yellowish in veins and reddish in arteries. Colloid and mucus stain blue.

The staining seems to follow any fixation well. Sections stained by this method have not faded in five years.

The writer states there is nothing new in the procedure other than the method of combining Delafield's hematoxylin with Mallory's connective tissue stain.

EXUDATES Classification Text for Exudates and Transudates D Allocco D Rif Med Naples 45 1209 1929

The following procedure is suggested as a means of differentiating between exudates and transudates.

To 4 or 5 cc of alcohol warmed in a test tube to 90° C the fluid to be examined is allowed to fall drop by drop. Exudates form at once an albuminoid coagulum which settles to the bottom of the tube later rising to the surface. Transudates form a powdery coagulum remaining on the bottom of the tube.

SCOPOMETRY The Junior Scopometer Exton W G J A M A 92 708 1929

Exton describes a new instrument for the measurement of turbidity and for a new method of colorimetry which uses the extraction point for both determinations.

The instrument is applicable to the determination of sugar in the urine, protein in blood, urine, spinal fluid and various other procedures.

AGGLUTINATION Stained Slide Microscopic Agglutination Test, Sabin, A. B. Am J Pub Health 19 1148, 1929

The following procedure is applicable to the rapid typing of pneumococci and the determination of antibody

The procedure for microscopic typing is as follows One cc of a fresh sample of sputum is injected intraperitoneally into a mouse, three to four hours after injection some of the peritoneal fluid is obtained by capillary puncture A glass slide is marked off into 4 parts, and a minute drop of the peritoneal fluid is expelled upon each one of the 4 partitions The first is smeared with saline for control, and the others with a loopful of a 1:10 dilution of Type I, of Type II, and of Type III, diagnostic serums respectively This dilution of serum is chosen largely to eliminate group agglutinins The smears are made thin so that they dry rapidly, they are then stained for one half minute with a fuchsin solution (10 cc saturated alcoholic solution of basic fuchsin plus 90 cc distilled water)

The stain is washed off in running water, and the smears are examined with the oil immersion lens If a specific agglutination reaction is observed in one of the smears with diagnostic serum, the organism is of the corresponding type If no reaction occurs in any of the smears, and pneumococci are clearly seen, a diagnosis of Group IV is made When it is desired to know whether the organism is one of the fixed types of Group IV, a similar procedure is carried out with the corresponding diagnostic serums Naturally occurring clumps of organisms differ in appearance from those produced by specific agglutination, they can be recognized by their occurrence in the saline control smear as well Unless a fresh sample of sputum is used, many of the organisms will have undergone autolysis and therefore more time must be allowed for growth Since the mouse is not killed, another typing can be done if the first one should show insufficient organisms, and after death of the mouse the type may be confirmed In the case of Type III, sufficient organisms for a microscopic typing are present as early as two hours after injection The appearance of the specific reaction with Type III differs from that obtained with other types of pneumococci, primarily on account of the larger size of the capsule

The technic for carrying out the microscopic antibody test is as follows

A drop or more of the patient's blood is taken with a capillary This is either centrifuged or, after coagulation and syneresis, a minute amount of serum is smeared with a loopful of a heat killed, saline suspension of the type of pneumococci for which it is desired to determine agglutinins The drops are thoroughly mixed and smeared very thin, allowed to dry in air, and stained for one half minute with the fuchsin solution recommended for the microscopic typing test The specific agglutination reaction is obtained here, but the clumps tend to be smaller

RECTAL CULTURES A Simple Method of Making Cultures of the Rectum, Traut, E F, and Herrold, R D J Infect Dis 45 172, 1929

The technic is as follows Vaseline is sterilized in small individual containers for stock use and finger cots are sterilized by boiling just before use At first the external surface of the skin and mucous membrane was cleansed thoroughly with soap and water Carefully repeated cultures proved this to be unnecessary if the external surface is coated with vaseline before the insertion of the finger Control cultures were made of the vaseline, fingers cots and external surface of the skin and mucous membrane Inoculations are made on ascites blood agar plates and the original inoculum streaked out in the usual way Cultures have been also without ascites to make certain that the ascites fluid was not bactericidal No difference could be noted between cultures made on ascites blood agar and those on plain blood agar The plates without ascites were made with 1 per cent agar The more luxuriant growth on the softer mediums is striking regardless of the bacteria implanted

Massage of the rectum with sterile, vaseline coated finger cot or glove, followed by inoculation of its tip onto blood agar plates, gave cultures of streptococci and other

bacteria in larger numbers as compared with the colon bacilli than could be secured by making cultures from the feces. The persistence of similar bacteria on repeated culture would indicate that the organisms found are not accidental saprophytes. A comparison of the fermentations by the streptococci isolated on successive culture is further evidence of the reliability of the results. The method offers an easy means of studying the relations of pathogenic bacteria of the rectum to disturbances of the intestinal tract itself, and also to arthritis and other systemic diseases.

ARTHRITIS The Bacteriology of the Blood and Joints in Chronic Infectious Arthritis
Oecil R. L. Nichols E. E. and Stainsby W. J. Arch. Int. Med. 43: 571 1929

The authors present evidence supporting the conception of arthritis as a streptococcal infection.

1 Blood cultures The technique employed for blood cultures was an adaptation of that recommended by Clawson in his blood culture studies of rheumatic fever.

Twenty cubic centimeters of blood were taken aseptically from the arm with a Luer syringe placed in two sterile culture tubes and allowed to clot. Each tube was treated separately in the following way:

The tubes were centrifugated, and all the serum drawn off with a sterile pipette. The clot was then broken up in the original culture tube with a sterile glass tube $\frac{1}{4}$ inch (6.35 mm.) in diameter. The fragments of clot were drawn up in the same glass tube and transferred to a 3 ounce bottle containing 50 cc. of beef heart infusion broth with a P_H of 7.6 (0.5 per cent sodium chloride, 1 per cent peptone). The bottle was then put in the incubator at 37 C. and left there unopened for five days.

At the end of this time a tube containing 8 cc. of 15 per cent beef heart infusion agar was placed in the water bath and heated until the agar was completely melted. The tube was then partially cooled and 0.5 cc. of whole rabbit blood added to it. Finally, the tube was seeded with 0.1 cc. of broth from the original blood culture and the contents poured into a Petri dish. The culture was allowed to incubate for from twenty-four to forty-eight hours. Similar pour plate cultures were made every three to five days thereafter until the original blood culture had been in incubation for thirty days. If at the end of this time the subcultures were still sterile the sediment in the original blood culture bottle was drawn out with a sterile glass tube and centrifugated. After centrifugating, part of the sediment was examined by means of stained smears while the remainder was cultured part of it in fresh blood broth and part of it on blood agar plates. If these final cultures from the sediment showed no growth the blood culture was considered sterile.

All of these cultures and transfers were made under a hood in order to eliminate contaminations as far as possible. All contaminated cultures were discarded.

When colonies appeared on the plates they were transferred into blood broth and identified by the usual bacteriologic methods.

2 Joint cultures Cultures from joints were made in blood broth from synovial fluid or synovial membrane or from bony curettings removed from the joint at operation. When only tissue was available the material was put in a bottle containing blood broth and macerated with a glass rod. These cultures like the blood cultures were incubated for several weeks and subcultured from time to time.

APPENDICITIS The Histologic Diagnosis of Chronic Menstrual
Research 16: 1019 1929

In the histologic diagnosis of chronic appendicitis the three features that are of importance are the presence of marked fibrosis and thickening of the submucosa, eosinophilic infiltrations of the submucosa and fibrosis or cell infiltrations of the muscle coat. Each of these by itself is sufficient to warrant a report of chronic appendicitis.

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building,
Richmond, Va

*The Female Sex Hormone*¹

IN THIS volume are detailed the researches of the author begun in 1904 and continued to the present time upon the nature of the female sex hormone

Part I (142 pages) is concerned with the biologic, pharmacologic, and chemical phases of the subject, while Part II (132 pages) is devoted to a discussion of clinical investigations based upon the female sex hormone blood test, the technic and applications of which are presented in detail

The volume may be accepted as a full and comprehensive presentation of a subject at present under discussion. A list of 528 references is appended

Synopsis of The Practice of Preventive Medicine[†]

WHILE this volume is intended primarily for the use of students, it may be read with profit by the practitioner as well

It is based upon the fact that courses in preventive medicine occupying a separate place in the medical curriculum are usually designed to consider the subject from the standpoint of the community rather than the individual, and so come to be regarded by the student as a subject apart from the clinical branches of medicine

It may be said, also, that this viewpoint is too often carried over into the practice of medicine whereas every doctor should be an exponent of preventive medicine as applied to the individual

The whole field of medicine is briefly covered, presenting the composite ideas of the faculty and stressing, under each heading, the prophylactic measures that should help the doctor in protecting the health of his patients. The volume is interleaved for notes and presents a comprehensive survey of the subject, brief but to the point

The Treatment of Diabetes Mellitus[‡]

THIS is an exceedingly valuable book both for the physician and the diabetic

It presents in detail the plan of treatment used by Sansum and his collaborators

The material is presented in two parts—a Medical Section and a Dietetic Section, both of which represent a clear cut, intelligible discussion of a complicated subject all too often too little understood by both patient and physician

The volume can be most highly recommended

Three Minute Medicine[§]

A SERIES of short essays on varied medical subjects intended for popular consumption

*The Female Sex Hormone. By R. T. Frank. Gynecologist Mt Sinai Hospital New York. Cloth 324 pages 86 illustrations 36 graphs C. C. Thomas Baltimore Md

†Synopsis of the Practice of Preventive Medicine as Applied in the Basic Medical Sciences and Clinical Instruction at the Harvard Medical School. Cloth 194 pages Harvard University Press

‡The Treatment of Diabetes Mellitus With Higher Carbohydrate Diets. A Textbook for Physicians and Patients. By W. D. Sansum, P. A. Gray and R. Bowden. Cloth 309 pages numerous tables Harper & Bros New York

§Three Minute Medicine. Brief Essays on Popular Medicine. By L. R. Effler M.D. Cloth 452 pages R. G. Badger Boston

NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto

*The Volume of the Blood and Plasma in Health and Disease*¹

IN THE fifteen years which have elapsed since its introduction by Rowntree, Keith and Cernghy the dye method of determining the plasma and blood volume has been subjected to extensive study and in its present state may be accepted as a valuable clinical aid.

The present monograph from The Mayo Clinic presents a comprehensive and authoritative review of all that is known of this subject to date presented by the authors, not only to clarify the subject but to emphasize the clinical value of such studies.

A short historical résumé begins the volume followed by a discussion of the criticisms which have been made of the dye method some valid and others not and this is turned by a detailed description of the proper technique for its performance.

The normal values are then discussed. These it would appear lie between 40 and 60 cc of plasma per kilogram regardless of sex. Three new terms are suggested "normovolemia" (within the normal range) hypervolemia (increased blood volume) and hypovolemia (decreased blood volume).

The remainder of the volume discusses the findings encountered in various conditions: obesity, diseases of the blood, diseases of the spleen and liver, various types of edema, diseases of the vascular system, diseases of the endocrine glands and miscellaneous conditions. The clinical data is further discussed in a separate chapter.

In hypertension an increased blood volume does not seem to be of importance and despite the hypertension in glomerulonephritis the blood volume is decreased.

The outstanding finding is the vigorous effort made by the organism to maintain the plasma volume within narrow constant limits. It is therefore one of the most striking and significant constants of the body. While its true relation to and significance to disease remain to be ascertained the present volume constitutes a definite and valuable contribution to the subject and well repays study.

¹The Volume of the Blood and Plasma in Health and Disease. By Leonard G. Rowntree M.D. and George E. Brown M.D. Division of Medicine The Mayo Clinic and The Mayo Foundation Rochester Minn. with the technical assistance of Grace M. Roth. Cloth. 19 pages. Illustrated. Philadelphia & London W. B. Saunders Co. 1929.

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EDITORIALS

The Backgrounds of Biologic Therapy

IT IS a long step from the first report of Edward Jenner in 1798 on his results from cowpox vaccination, and from the studies of Pasteur in 1884 on the prevention of rabies, to our present-day immunologic understanding and practice. Were these two pioneers to revisit the world they would undoubtedly express the greatest astonishment at the tremendous advances that have been made.

And yet the question has been seriously raised of late, and quite rightly, whether our forward strides have been especially great, particularly when measured in terms of the clinical application of today's generally accepted immunologic principles.

Hektoen and Irons have tabulated the replies to a questionnaire on vaccine therapy which was answered by 1,261 practicing physicians, and of this number only seventeen physicians consider vaccine therapy to be a generally useful and superior method of treating infectious diseases. Four hundred and

thirty have never used autogenous vaccines in the treatment of any disease, 142 have used them rarely, and 172 report having abandoned the use of autogenous vaccines in therapy. Five hundred and seventy seven did not use or have never used stock polyvalent vaccines in the treatment of any disease, 49 have used them very rarely, 198 report having abandoned the use of stock polyvalent vaccines because of their failure as therapeutic agents.

The authors point out that from 1906 the date of Wright's introduction of vaccine treatment the number of papers on vaccines in medical journals constantly increased until it reached a formidable volume around 1912. Since then interest in the treatment has evidently declined as measured by the number of contributions in current literature.

Manwaring and Krueger calculate that our fundamental immunologic theories and current experimental laboratory methods have assayed less than 5 per cent clinically verifiable truth. Applying our present knowledge it would appear that of twenty theoretically logical laboratory endorsed vaccines and antiserums, only one would prove a clinical success.

The two preceding references constitute ample evidence that it is high time that we take inventory. At the same time, there is great danger of these writers being misquoted and misunderstood. Neither group has denied the remarkable advances that have been made in this field. Hektoen and Irons specifically state that they do not draw conclusions with regard to the value or lack of value of vaccines in treatment. Their one interest has been to ascertain what opinion practicing physicians have formed of this method and how that opinion has affected the present use of vaccines. Indeed if they had discovered enthusiastic proponents of vaccine therapy in some of the diseases which they tabulate such as appendicitis, diabetes, nephritis, dermatitis, eczema, focal infection, gall bladder infection, gastric ulcer, hay fever, meningitis, peritonitis, and epilepsy, we would be forced to conclude that the physician of today was using extremely poor judgment in his clinical application of our immunologic theories.

Manwaring and Krueger, far from being discouraged, remark that modern immunology is justly proud of its 5 per cent clinical efficiency, the high watermark of all history. One need only recall by way of comparison the 2 per cent verifiable clinical value of the fundamental biologic theories of the mid Victorian era and the 1 per cent clinical coefficient of Aesculapius.

However, it is really a question whether the average physician is as critical in his actual practice as he is in his replies to questionnaires. As Kinsella remarks, one has only to compare the catalogue of any prominent drug company of twenty years ago with their most recent list to realize that the treatment of infectious diseases has been changed from one in which drugs played the most important part to one in which such biologic products as serums and vaccines have assumed a great prominence.

This condition reflects an attitude on the part of the physician which is due to the lack of uniform teaching regarding the nature of infection, its etiology and the means by which an infectious disease may be neutralized. The medical student hears contradictory evidence in many fields of bacteriology. At the same time the information which he receives concerning anti-

gens and antibodies is so rigidly definite that he leans to a belief in specificity and is inclined to accept as specific the various agents for immunization, however loosely supported they may be by the uncontrolled observations of clinicians or the claims of salesmen."

What have we to show for the 5 per cent clinically efficient immunologic remedies? Smallpox vaccine, rabies vaccine, typhoid vaccine, diphtheria toxin-antitoxin, diphtheria antitoxin, tetanus antitoxin, antipneumococcus serum Type I, antimeningococcus serum, streptococcus scarlatinae antitoxin, poliomyelitis immune serum possibly cyvisipelas antitoxin, antivenin, pollen and other allergenic extracts, the Wassermann test, the Widal test, and similar precipitin and agglutination tests. Remove all of these and the armamentarium of the physician would be sadly depleted. The modern specific treatment of the allergic diseases is as directly the outgrowth of our present understanding of the phenomena of anaphylaxis and immunity as is the case with any other biologic measure employed in modern medicine. And 66 per cent of relief in hay fever, 50 per cent in allergic eczema, and 37 per cent in migraine by specific measures only, are truly substantial figures.

The concepts of a hundred years hence will no more resemble those of today than do our present concepts resemble those of Galen which controlled medicine for 800 years. And yet the wonder is that we have been able to accomplish so much by the clinical application of what little today is known in the field of immunology. Our indebtedness to the pioneers of the last half century is great indeed. Let us glance back for a moment at the historical background in one section of this broad field.

ANAPHYLAXIS

As early as 1839 Magendie had noticed that rabbits which had received without untoward symptoms a first injection of albumin could not sustain the same dose several days later. In 1888 Victor C. Vaughan observed that laboratory animals which had once been inoculated could not safely be used for subsequent experimentation. In the standardization of diphtheria antitoxin it soon became evident that guinea pigs that survived one test could not be relied upon in a second one. In the late nineties Parke-Davis and Company, aware of this fact, offered to supply the Hygienic Laboratory of the University of Michigan with "used pigs" at a small price. The offer was accepted but the animals were found to be high at any price, as they suddenly and unexplainably died when used in experimental work.

Undoubtedly many observers of that time were aware of this unusual manifestation, for several years later Theobald Smith mentioned the phenomenon to Ehrlich, who set his student Otto to work to find the explanation. Otto in publishing his findings described this phenomenon as the *Theobald Smith Phenomenon*.

Flexner in 1894 observed that rabbits which had received dog serum without untoward effect died promptly after a second dose several days or weeks later. The second dose was often smaller than the first. Behring at about the same time (1893) also observed the curious results of reinjection of

diphtheria antitoxin in the guinea pig Working with him Knorr and Kitasato determined that the second dose might be fatal even though it was seven or eight hundred times smaller than the initial dose Behring described this as a paradoxical reaction

In 1894, again Arlong and Courmont observed that successive injections of donkey serum into man produced increasingly toxic effects Richet in 1898 while studying the effects of eel serum on dogs often observed death after the second or third injection Courmont noticed in 1900 that successive small doses of tuberculous pleural exudate caused guinea pigs to die before having received a quarter of the total dose which they could easily withstand in a single injection

Thus we see that between 1839 and 1902 the phenomenon that we now designate anaphylaxis had been observed often enough but no attempt had been made to study it farther or to explain it recourse merely being had to such terms as atypical or paradoxical reactions It remained for Richet and Portier to make a really systematic study of this phenomenon and to offer a tentative explanation Their communication was first published on February 15 1902 and it was on this day that allergy or anaphylaxis as we understand it now, first saw the light of day

Richet and Portier who were watching as guests of the Prince of Monaco in the southern seas inaugurated at the latter's suggestion a study of the toxic properties of the *physalia* commonly known as Portuguese Man o' War These like our own jelly fish produce an intense skin irritation on contact On their return to France they were unable to obtain a further supply of *physalia* so they determined to carry on their experiments with the *sea anemone*

Making glycerine extracts of the tentacles they attempted to determine the toxic dose of the irritating substance Just as with diphtheria antitoxin dogs that survived were held over for later testing

It was a dog which answered to the name of Neptune whose contribution to humanity was the opening of the portals of the science of allergy Neptune, an unusually healthy and robust dog received 0.1 cc of glycerine extract of *anemone* hypodermically, without becoming ill Twenty two days later while still in the best of health Neptune received another dose of 0.1 cc A few seconds later he became extremely ill with extreme asthenia dyspnea diarrhea and bloody vomiting He soon became unconscious and was dead within twenty five minutes

Richet's subsequent studies and interpretations as summarized in his theory of anaphylaxis published in 1902, presented an entirely new understanding of these paradoxical reactions The keynote to the phenomenon lay in two facts first, that a substance which an animal could receive with impunity might on re-inoculation become most highly poisonous, and second that a certain amount of time must elapse between the two inoculations for the toxic change to become manifest

Space does not permit a review of Richet's theory Suffice it to say that evidence was presented that the introduction of a foreign protein even in

small quantity into a living animal produced profound changes in the tissues of the inoculated animal

This observation was at once utilized to explain the tuberculin skin reaction which had been described by Koch in 1890. The tuberculin reaction was now accepted as evidence that a profound change had occurred in the tissue of the human organism as a result of the presence of the tubercle bacillus protein.

Victor C. Vaughan in the meantime had been carrying on research into the nature of bacteria and bacterial infection. As early as 1890 he had demonstrated that the bacterial cell contains or metabolizes a poisonous substance and that this substance is the same or similar in different types of bacteria. Following Richet's announcement he at once applied these observations to his studies of bacterial infection and was able to confirm Richet's findings. He next demonstrated that the poisonous fraction which he was able to separate from the protein molecule appeared quite the same or similar in such diverse proteins as bacteria, horse serum and egg albumin. On the basis of this and further observations he evolved his own theory of protein sensitization which was presented in its completed form in 1906. He applied this knowledge in the development of his theory of bacterial infection and immunity.

Richet proposed the term anaphylaxis, meaning "without protection," as an interpretation of his observation that animals in whom he was building up protection by injections sometimes apparently lost this protection completely, becoming abnormally sensitive. Vaughan and Wheeler demonstrated that the same fundamental process was at work, both in immunity and in anaphylaxis, and to this extent the term anaphylaxis is a misnomer. Today we might with equal reason use the term hyperphylaxis for the same phenomenon. Von Piquet proposed the more acceptable term, allergy, in 1906, meaning altered energy, or altered reactivity, expressive of the deep-seated systemic changes which have taken place within the organism following a first introduction of foreign protein. It is this term which has been adopted in clinical work. Anaphylaxis is still used in clinical medicine but usually to express that fulminating reaction occasionally observed after parenteral inoculation, which presents a picture altogether similar to that seen in experimental animals and is known generally as anaphylactic shock.

Many other theories of anaphylaxis have been proposed but it is safe to say that subsequent developments and our present knowledge are founded on three theories, the Ehrlich side chain theory from Germany, the Richet anaphylaxis theory from France, and the Vaughan protein poison theory from America.

These three theories were offered to explain phenomena that were known to exist at that time. Further laboratory investigations have produced many phenomena which cannot be satisfactorily explained by any one or all three of the theories, and which probably eventually will call for their reconstruction or discard. However, in the meantime we have traveled surprisingly far especially in the field of clinical allergy with the aid of these hypotheses.

As Victor Vaughan remarked in 1893, "The value of a theory does not wholly depend upon its truth, but is rather to be measured by the fruitfulness of the lines of investigation that it opens. Indeed a theory may be wholly erroneous and yet it may lead to most important discoveries."

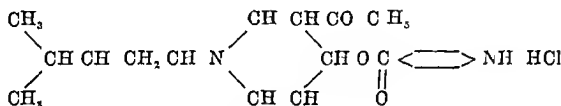
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Erratum

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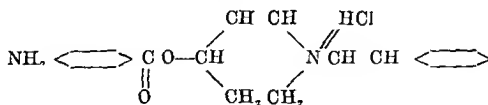
Chemical name for No 33 G should be written as

Gamma (2 methyl piperidino) propyl benzoate HCl

On page 242, one of the hydrogens should be removed from the beta carbon of formula No 56 to which the methyl group is joined

Formulas Nos 92 and 93 should have double bonds between the two CH groups

Formula No 58 should be written as



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In the latent phase patients present no symptoms of anemia but give a history of rheumatic attacks, pain in the epigastrium and left hypochondrium and periods of weakness and dyspnea which have been separated by years of normal health. Fresh blood preparations may show a few or no sickle cells, but upon standing many cells assume the sickle form.

Sydenstricker was also the first to describe in detail the pathologic findings in this condition. The most important findings are revealed in the study of the blood, liver, spleen, and bone marrow. The blood shows the characteristic fusiform and sickle shaped erythrocytes. The liver is hypertrophied and shows evidences of cloudy swelling with non-free pigment in both the liver cells and the Kupffer cells. The spleen is firm and on section the cut surface is very dark red with no visible lymphoid nodules. Microscopically the spleen seems overfilled with blood, the trabeculae are prominent, and the small malpighian bodies are surrounded by areas of intense congestion. The sinuses and the spaces of the pulp are engorged with blood. The endothelial cells of the sinuses are heavily laden with brown iron-free pigment. The bone marrow is usually abundant, bright red, and thin in consistency. The reticular spaces are filled with erythrocytes intimately mingled with leucocytic constituents of the marrow. Large clusters of sickle shaped erythrocytes may be seen between the capillaries.

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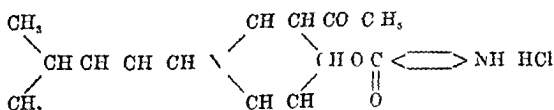
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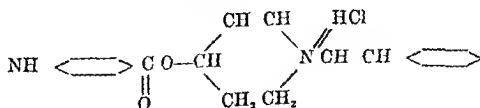
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CLINICAL AND EXPERIMENTAL

SICKLE CELL ANEMIA*

By WILLIAM Z. FRADKIN, A. B., M. D., AND LEO S. SCHWARTZ, M. D., F. A. C. S.
BROOKLYN, N. Y.

SICKLE cell anemia is a hereditary blood dyscrasia with an unusual extensive symptomatology. Because of the latter surgeons often mistake this disease for an acute abdominal condition, or clinicians for acute hemolytic icterus or syphilis. It is only by the accidental discovery of sickle cells in the routine examination of the blood smear, that the diagnosis is made. In this paper it is our intention to review the literature and report a case of sickle cell anemia with rapid improvement, following laparotomy and transfusion.

Sickle cell anemia was first discovered by Herrick in 1910. Up to the year 1923 only four reports of similar cases appeared in the literature. Mason was the first to use the term "sickle cell anemia" in his report of the fourth case. Sydenstricker was the first to really stimulate interest in this disease. His papers read before the members of the Southern Medical Association and the American Medical Association in 1923 and 1924 were the most accurate and scientific presentations of the time, and established sickle cell anemia as a definite clinical entity with a characteristic blood picture and a definite pathology.

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From The Gynecological Service of Dr. Schwartz of The Jewish Hospital of Brooklyn.
Received for publication September 4, 1929.

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News and Notes

The Ninth Annual Convention of the American Society of Clinical Pathologists will be held in Detroit, Michigan, June 20, 21, and 23, 1930. The Book Cadillac Hotel has been selected as our official headquarters and the management assures us that all reservations if so desired will be carried on through the meeting of the American Medical Association the following week. You are, however, urged to make your reservations as early as possible in order that you may secure the exact accommodations you want.

The scientific meetings of the Society will be held in the Crystal Ballroom of the Book Cadillac Hotel with the scientific and commercial exhibits in the adjoining Italian Garden.

Fellows of the American Society of Clinical Pathologists are invited to reserve space for a scientific exhibit at Convention and to enter the contest. A Gold and a Silver Medal will be awarded to the members presenting the best scientific exhibits. This should be a stimulus for a very interesting and instructive display.

If you wish to present a scientific paper you may communicate with the Secretary, Dr H J Corper, 256 Metropolitan Building, Denver Colorado, giving the exact title and, if possible, an abstract.

Reservations for hotel accommodations and space in the scientific exhibit may also be made through the Secretary.

The plans made by the Local Committee on Arrangements under the Chairmanship of Dr F W Hartman insure a very successful and enjoyable convention for all those in attendance. Lock up your workshop and attend the Detroit Convention to procure your annual scientific stimulant.

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2 If normal red cells of the same blood group as the patients are washed in isotonic salt solution and suspended in the patient's serum the normal cells will remain unchanged

3 Preparations of blood from cases of pernicious anemia, secondary anemia, myeloid and lymphoid leucemia congenital hemolytic jaundice, and Banti's disease show no change in their morphology after standing for one week

4 Intravenous injection into rabbits of a 50 per cent suspension of washed red cells of a patient with severe symptoms of the disease provoked no visible abnormality in the blood of these animals

5 Repeated blood cultures are negative in patients with sickle cell anemia

Graham reported a case of sickle cell anemia with necropsy and pointed out that the red cells of the tissues fixed in formaldehyde were all sickle cells, but those of the tissues fixed in Zenker's solution were normal in shape

Moser and Shaw and Anderson in their report of one case each in Northern negroes, showed that this disease was not peculiar to the Southern or Tropical negroes

Sickle cell anemia may be complicated by gallstones in which case the differential diagnosis is extremely difficult, clinically, because of the presence of gall bladder symptoms similar to that of gall bladder pathology. Such a case was reported by Hamilton

Cooley and Lee found sickle cells present in the bloods of $7\frac{1}{2}$ per cent of the 400 patients admitted to their clinic during a period of ten months. From their studies of the phenomena of sickling they conclude the following

1 In preparations kept at incubator temperature the sickle cells disappear rapidly from the blood

2 The cells of one of the patients with sickle cell anemia were rapidly hemolyzed at incubator temperature in their own serums and in serums from normal bloods while the serum of this patient was not hemolytic for normal cells

Alden in his report of two cases emphasized the fact that sickle cell anemia may be confused with tertiary syphilis due to the frequent presence of leg ulcers. In his second case the pain in the abdomen and sudden distention were thought to be due to an acute appendicitis but in all probability were due to a splenic hemorrhage

Josephs examined 14 samples of blood from patients with sickle cell anemia by thoroughly washing the red cells in salt solution and succeeded in removing an unknown substance which is responsible for the sickle cell formation

In a paper on the origin and fate of sickle shaped red blood cells Levy described peculiar changes in the red cells. Embryonic red blood cells and normoblasts divide or extrude their nuclei. The resultant cells project pseudopodia and assume bizarre shapes. Heat hastens the change and cold inhibits. Some of the abnormal red blood cells retract their pseudopodia and return to the parent form while others retain their bizarre shapes permanently. Sickle cells kept at body temperature eventually disintegrate by fragmentation

Hahn and Gillespie were the first to attempt splenectomy for the treatment of active sickle cell anemia. The patient showed evidences of great blood de-

struction with a rapid drop in the red cell count. A splenectomy was followed by marked clinical improvement with a rapid return of the red cell count to almost normal figures. These authors also report a series of experiments in which various gases were passed through a chamber containing a suspension of a patient's red cells. They showed that the red corpuscles of persons with the "sickle cell trait" are transformed into sickle cells *in vitro* as a result of asphyxia. The transformation takes place when the oxygen tension falls below a partial pressure of 45 mm of mercury provided the hydrogen-ion concentration is within certain limits, probably always on the acid side of P_H .



Fig 1—Stained blood smear

74 Oxygen and carbon monoxide induced restoration of the discoid form. They are of the opinion that sickle cell formation *in vivo* is probably induced or increased by anoxemia.

Hem, Gordon, McCalla and Thorne placed red cells of their patient with sickle cell anemia in sera, of his own mother, of a normal negro, of a patient with pernicious anemia, of a patient with acquired type of hemolytic icterus, of a patient with obstructive jaundice, and noted the development of the typical deformity in the cells. Concisely, the red cells of these people when placed in the serum of the affected negro boy showed no sickling.

Stewart observed a case of sickle cell anemia in a child six years of age, neither of the parents were of direct negro descent. It is the only case on

record in an apparently white child of Cuban descent. The spleen was observed by the author in its transition from a hypertrophic to an atrophic organ. A splenectomy was performed with a seemingly rapid but uneventful recovery.

Bell, Kotte and Mitchell reported a case of sickle cell anemia in a colored boy aged eighteen months where splenectomy did not affect the sickle cell picture nor lessen the existing marked anemia. Cooley and Lee suggested an explanation for the latter by stating that a supernumerary spleen might have been overlooked at the operation.

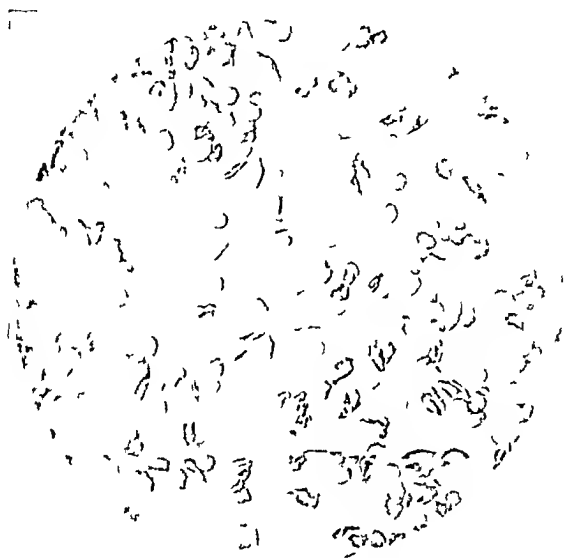


Fig. —Sealed preparation of blood after twenty four hours

Wollstein and Kreidel reported two cases of sickle cell anemia in colored children whose cause of death was apparently due to the anemia itself, for at autopsy no other cause of death was demonstrable.

It is interesting to note that Rich in a study of the sections from 5000 consecutive autopsies was able to demonstrate a characteristic splenic lesion in 62 cases. The specific histologic appearance consisted of a pronounced malformation of the sinuses immediately about the malpighian bodies leading to the formation of pools of blood partially or completely surrounding the malpighian bodies. Sickle cells were found in the blood in the histologic sections in every case. Immediate relatives of these 62 autopsied individuals were examined by Josephs and in each case the sickle cell trait was found.

Bennett was able to recognize sickle cell anemia from necropsy material. He crushed blood clot from tissues hardened in 10 per cent formaldehyde, in physiologic solution of sodium chloride, and made smear preparations from the resultant suspension. Microscopic examination of these smears showed the typical sickle cells. The lapse of time after fixation seems to be of no importance in the amount or character of the red cell distortion.

REPORT OF CASE

E. R., a colored female, aged twenty-six, married, and residing in Brooklyn for the past three years, but born and reared in South Carolina, came to the

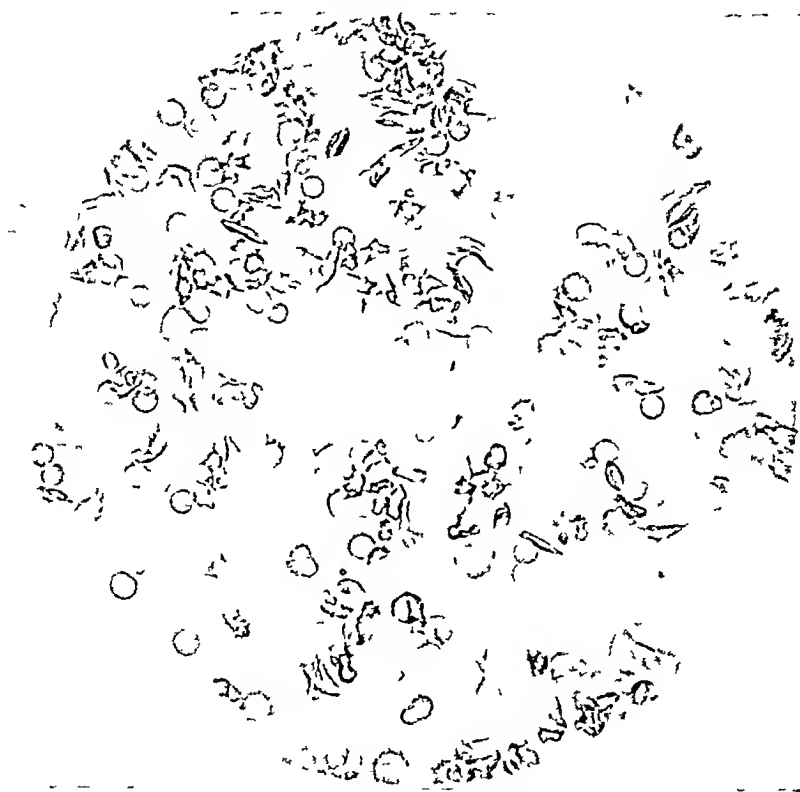


Fig. 3—The same blood preparation after forty-eight hours

Medical Clinic of the Out-Patient Department of the Jewish Hospital on March 13, 1929, complaining of pain in the abdomen.

A medical condition being ruled out, the patient was referred to the Gynecological Clinic. On examination a diagnosis of acute adnexitis was made and the patient was admitted to the Gynecological Service of the same hospital on April 4, 1929.

Her chief complaint was pain in the abdomen. The family history was negative. During her childhood the patient had measles, whooping cough, chicken pox, and smallpox. At the age of nine she had "rheumatism" with recurrent attacks of pains and fever up to the age of fifteen. Since the age

of fifteen, the patient states that she feels pains in the joints and muscles occasionally. Had malaria at the age of thirteen. Denies venereal infection. Has a negative surgical history. Marital history irrelevant. Menstrual history negative. Patient has had dyspnea and palpitation on exertion for many years. Has always had frequent spells of dizziness. Except for the pains in the extremities and back the patient was feeling generally quite well up to the present illness.

The present illness dates back to ten weeks ago when the patient was seized with dull aching pain localized in the lower abdomen more severe in the right lower quadrant. There was no nausea or vomiting present. Patient had fever and a yellowish white vaginal discharge since the beginning of the illness. On the day of admission the patient also developed some epigastric pain with tenderness and rigidity over the right and left upper quadrants especially on the right side. Appetite was fair with only slight distress after meals. Constipation was present. Slight dysuria but no frequency.

Physical examination revealed a colored female well developed but poorly nourished anemic and appearing acutely ill with a temperature of 102° F. Eyes were prominent but showed no other signs of exophthalmic goiter. Right eye showed a corneal scar adherent to the iris. Both sclerae were greenish yellow in color. Pupils were equal and reacted to light and accommodation. The palpebral conjunctivae were very pale. Nose and ears were negative. The mucous membrane of the mouth was very pale. Tongue slightly coated, teeth in fair condition. Tonsils submerged but cryptic. Neck revealed small palpable cervical lymph nodes. The chest showed evidences of early rickets. The lymph nodes in the axillary fossae were palpable.

Examination of the heart revealed a soft systolic murmur at the apex and along the left border with a loud systolic over the aortic and pulmonary areas.

The lungs revealed occasional subcrepitant rales at extreme right base posteriorly.

The abdomen was tense, doughy with resistance and tenderness over the right upper quadrant and somewhat over the left hypochondrium. There was also some tenderness at McBurney's area. The liver was palpable 3 cm below the right costal margin. The spleen was not felt. Kidneys were not felt.

The upper extremities were negative. The tibiae of both lower extremities were prominent and showed large scarred areas over the middle third of the anterior surfaces. Reflexes were apparently normal.

LABORATORY FINDINGS

Blood Picture—

Hemoglobin (Dare)	36 per cent
Red blood cells	~ 300 000
Color index	0.78
Bleeding time	2 minutes 45 seconds
Coagulation time	6 minutes 0 seconds
Platelets	120,000
Total white blood cells	12 400
Polymorphonuclears	83 per cent

Laboratory Findings

Blood Picture—Cont'd

Eosinophiles	1 per cent
Basophiles	0.75 per cent
Lymphocytes	38.0
Turb cells	1.0
Monoocytes	5.0
Promyelocytes	0.50
Neutrophilic myelocytes	0.75
There were 2 nucleated red blood cells in the 400 cells counted	
Marked oligochromasia	
Occasional stippled red blood cells	
About 8 per cent of stained red blood cells were sickle shaped (Fig. 1)	
Fresh preparation shows 25 per cent sickling and 98 per cent sickling after twenty four hours. All red blood cells rounded after seventy two hours (Figs. 2 and 3)	
Marked anisocytosis	
Poikilocytosis	
Macrocytosis and polychromasia	
1 per cent normoblasts per 100 white blood cells	
14 per cent reticulation	
Supravital staining showed one mononuclear small sized white cell which has phagocytosed a red blood cell	
Red blood cells showed increased resistance	
Hemolysis began at 0.34 per cent saline and was still incomplete at 0.28 per cent saline	
Sedimentation time	18 minutes
Wassermann	Negative
Kahn	Negative
Bilirubin	Negative
Direct	1 unit
Indirect	
Icterus index	12
Sugar	115 mg per 100 cc
Urea	7.8 mg per 100 cc
Creatinine	1.6 mg per 100 cc
Cholesterol	238 mg per 100 cc
Chlorides	625 mg per 100 cc
Calcium	9.2 mg per 100 cc

Renal Function (Phenolphthalein)—

First hour	56 per cent
Second hour	19 per cent
Total renal function	75 per cent

Urine—

Specific gravity	1.010
Reaction	Acid
Albumin	Faint trace
Sugar	0
Microscopic	Occasional white blood cells
Urobilinogen	1500 dilution units
Urobilin	2700 dilution units

Stool—

Bile	Present
Blood	Negative guaiac
No ova or parasites found	
Urobilinogen	4800 dilution units
Urobilin	14,400 dilution units

Gastric Analysis—

Free hydrochloric acid	2 per cent
Total hydrochloric acid	3 per cent
Lactic acid	Negative
Microscopic	Negative

Vaginal Smear Negative for gonococci

Cervical Smear Negative for gonococci

Vaginal examination revealed the uterus to the left, retroverted, and a fixed insensitive cystic mass in the right and posterior fornices

The patient's temperature fluctuated from 100° to 103° F seldom remaining low for a whole day at a time

The patient's mother, brother and two sisters were examined and none of them showed a condition similar to that of the patient Fresh and old blood preparations both dry and wet showed no active or latent sickling of the red blood cells

The patient did not respond to medication and nonspecific protein injections A surgical consultation was requested in order to rule out a possible acute abdominal condition A diagnosis of acute cholecystitis was made, and the patient was therefore transferred to the surgical service On opening the abdomen the liver was found adherent to the abdominal wall The peritoneum was studded with tubercles varying in size from a millet seed to a pea All of the small intestine was covered with minute tubercles Many of the loops were adherent The gall bladder was negative The spleen was small, covered with tubercles and adherent to the anterior abdominal wall The pelvis was found walled off by adhesions, and the tubes were probably the site of tuberculous invasion The mesenteric lymph nodes of both small and large intestines were enlarged to the size of a walnut

Microscopic examination of a mesenteric lymph node revealed active tuberculosis

The patient became very weak following laparotomy A blood count revealed 2,200,000 erythrocytes and 34 per cent hemoglobin (Dare) An intravenous transfusion of 500 cc of blood was given on May 15, 1929, with prompt benefit The general condition of the patient began to improve steadily, the temperature dropped to normal, the abdominal tenderness and rigidity disappeared the abdominal wound healed rapidly and the patient was discharged on June 2, 1929, nineteen days following operation

COMMENT

This patient complained of pain in the lower abdomen, fever and vaginal discharge After admission she also developed epigastric pain with tenderness and rigidity over the right and left upper quadrants It was thought that the patient had a subacute pelvic peritonitis in addition to the already existing adnexitis The sudden onset of upper abdominal symptomatology however made a diagnosis of an acute cholecystitis possible Although a diagnosis of sickle cell anemia was made during the first week of the patient's stay in the hospital, its significance was not realized until after operation Pain, tenderness and even rigidity over the gall bladder region are very common symptoms and signs in cases of sickle cell anemia

Patients with sickle cell anemia are very susceptible to tuberculous infections and in this case a diagnosis of tuberculous adnexitis should have been considered In fact, over 50 per cent of the reported cases of sickle cell anemia succumbed to tuberculosis This patient had an apparently negative chest on x-ray examination Dolgopol and Stitt found the sickle cell phenomenon in 52 per cent of the 77 tuberculous patients they examined Tuberculosis

not therefore be considered as an etiologic factor in the development of sickle anemia, but rather a complication as a result of the lowered resistance of the patient

Clinically, sickle cell anemia closely resembles familial hemolytic jaundice. The latter condition however shows an increased fragility of the red blood cells, an enlarged spleen and absence of leg ulcers or scars, joint pains and crises of abdominal pain.

It is interesting to note that in this case the direct Van den Bergh was negative, the indirect only 1 unit while the icterus index was 12 with a marked increase in the urobilin and urobilinogen of the urine and stool.

Upon careful questioning of the patient regarding the scars over her legs she stated that she had blisters over the legs as the result of sitting barelegged in front of the fireplace during the winter months of her childhood, and that those blisters broke leaving scars over her legs. This is interesting for there is no such explanation as far as we know in the literature.

CONCLUSIONS

- 1 A complete review of the literature of sickle cell anemia is given.
- 2 A case of sickle cell anemia complicated by mesenteric tuberculosis is reported with improvement following laparotomy and transfusion.
- 3 A diagnosis of an acute surgical abdomen in a negro patient is hazardous without a previous search for sickle cells in the blood smear.
- 4 Emphasis is directed to the fact that most patients with sickle cell anemia are susceptible to tuberculosis probably because of their lowered resistance to infection.
- 5 A rather simple explanation is given for the leg ulcers or scars so often encountered in patients with sickle cell anemia.

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SPONTANEOUS MENINGEAL HEMORRHAGE^a

BY FREDERICK H LAMB, M D, DAVENPORT, IOWA

CASE 1—About ten years ago with one of my confreres, I saw a twelve year old girl, who had been seized sixteen hours previously with a sudden sharp, excruciating pain in the head. She had been playing the piano, when she stopped suddenly, ran to her mother with her hands clasped tightly to her head. She screamed with pain, which seemed quite undurable, soon became irrational, vomited, and tossed about on the bed, crying out, and moaning in agony. Presently she lost consciousness completely.

When seen fourteen hours later, the child was profoundly comatose. Her pulse was 60, temperature 100.5° F, respirations slow and labored. The pupils were equal and dilated, reacted incompletely to strong light, there was a spontaneous lateral nystagmus, the conjunctivae were injected. She had had involuntary micturition and defecation, and a brisk epistaxis lasting about twenty minutes. There was a definite general rigidity of the body. The leucocyte count was 14,200. The urine contained a trace of albumin, but no sugar, acetone, diacetic acid, nor casts.

There was nothing significant in the child's family, nor her own medical history. No one knew of her having had any injury to the head.

A lumbar puncture yielded 60 cc of uniformly bloody fluid under greatly increased pressure. On standing a half hour, the red cells had settled in the test tube so that the upper fourth of the contents was water color, and at the end of twenty four hours at 8° C, the cells had settled down to about one tenth the total volume, with no sign of clotting. Smears and cultures were bacteria free.

Following the spinal drainage, the patient's general condition began to improve. After a second withdrawal of fluid, her condition warranted the hope of recovery. She improved steadily, and at the end of two weeks, seemed to have quite fully recovered from her experience. She is now a healthful young woman.

This was my introduction to that condition known, perhaps for want of a more significant name, as spontaneous meningeal hemorrhage (although at that time I believed it to be the clinical picture and symptom complex of non-traumatic pachymeningitis hemorrhagica interna). That there is a similarity in these two conditions might be deduced from a superficial scanning of the literature, yet a more careful study will bring out distinctions, which deserve a much more general appreciation.

INCIDENCE

It is difficult sometimes to know whether a disease is occurring more frequently or whether it is being recognized more commonly. Certainly, as Josephine B. Neal¹ says, there are but brief references to spontaneous meningeal hemorrhage in English, although in France and Sweden, a rather extensive literature has been developed.

My experience with 7 proved cases, and 2 probable cases, within a ten year period leads me to think that the condition is overlooked more fre-

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quently than it is diagnosed. It has, furthermore, pointed out the need of differentiating this condition from certain other intracranial hemorrhagic conditions.

PATHOLOGY

Let us say at once, that we are not concerned with those hemorrhages occurring as a complication of such conditions as pyogenic or tuberculous meningitis, arthritis, septicemia, pertussis, smallpox, anthrax, nephritis, uremia, eclampsia, insolation, caisson disease or aviator's sickness. Neither are we concerned with the hemorrhagic diatheses scurvy, purpura, hemophilia, nor leucemia and only indirectly with lead, alcohol, syphilis, and arteriosclerosis in so far as they may be contributing factors in individual cases. In most instances of spontaneous meningeal hemorrhage, these factors have no bearing whatever.

We cannot, however, dispose so readily of the sequelae of cranial trauma, nor of congenital aneurysms near the circle of Willis as etiologic factors. We are directly concerned in the differential diagnosis with subdural hematoma in its various stages of development, also, with the similar if not identical condition pseudomembranous pachymeningitis hemorrhagica interna.

To review briefly the latter condition on account of its close relationship to the subject of this paper, there is a growing belief that chronic subdural hemorrhage, and the pachymeningitis hemorrhagica interna of Virchow² are one and the same.³ It is interesting to note that chronic subdural hemorrhage is of venous origin thereby differing from all other forms of intracranial hemorrhage which are generally the result of arterial rupture.⁴ The pathologic picture as found at operation or autopsy—and certain of the clinical symptoms are admirably explained by the mechanism of forming the subdural hematoma. According to Holmes⁴ and others, the hemorrhage is supposed to result from the tearing of small veins which enter the tributaries of the superior longitudinal sinus. At the point at which the superior cerebral veins enter the sinuses, the arachnoid is firmly adherent to the dura mater. This anchorage evidently protects the large veins, and the sinus itself, while the small vessels are stretched and torn by displacement of the brain resulting from traumatic force. The ultimate result is a more or less constant seepage of blood with organization of the clot more or less in keeping with the age of hematoma.

DIFFERENTIAL DIAGNOSIS

From a clinical standpoint, it is desirable to recognize the extradural hemorrhage, due for example to rupture of the middle meningeal artery, and an encysted subdural hematoma referred to above following more or less remotely a blow on the head. Direct surgical relief is possible in both conditions. It is also desirable to distinguish between these two conditions, as a group, and the primary subarachnoid hemorrhage due, for example, to the rupture of a circle of Willis aneurysm, or the primary cerebral hemorrhage with or without extension into the subarachnoid space. Neither of the latter conditions is amenable to surgery.

Briefly stated, the clinical syndrome of spontaneous meningeal hemorrhage is the picture of severe meningeal irritation with a "brutal onset," and without obvious cause, the uniformly bloody spinal fluid under increased pressure, and usually prompt relief of symptoms following removal of fluid.

On the other hand, the symptomatology of chronic subdural hemorrhage is extremely variable. According to Holmes, there is the history of injury, the gradual onset of headache, sleeplessness, forgetfulness or vertigo. There may be a general lowering of efficiency, general stupidity, irritability, often a thick aphasic speech. The second stage is characterized by a sudden aggravation of symptoms. The headache becomes worse, the drowsiness passes into stupor, and periods of consciousness alternate with periods of unconsciousness. Finally a moderate hemiparesis develops due to unilateral cerebral compression, although a frank hemiplegia is rare.

The symptoms of intracerebral or capsular hemorrhage are too well known to reiterate here. The association is between coma and paralysis, instead of coma and paresis, and the history of the two conditions is unlike.

SPINAL FLUID

In connection with the clinical symptoms of spontaneous meningeal hemorrhage, the most valuable single finding is the character of the cerebrospinal fluid. A uniform and decidedly bloody fluid under pressure is evidence of gross hemorrhage into the subarachnoid space. It is the cardinal sign of spontaneous meningeal hemorrhage. It is very much less likely to be found in either the typical case of extra, and subdural hemorrhage, or intracerebral hemorrhage. Conceivably both of these conditions may advance to the point of blood escaping into the subdural space, and the finding of a few erythrocytes in microscopic examination, or presence of xanthochromia is not infrequently observed. But the presence of a large amount (7 per cent, or more) of blood in fluid under greatly increased pressure is a strong point in favor of meningeal hemorrhage. Furthermore, if the fluid be recovered within twelve to twenty-four hours after the onset of symptoms, it will be observed that on standing, the supernatant fluid is water color. Succeeding withdrawals of fluid will show an increasing xanthochromia, with or without a diminution in the number of red cells.

It is worth while noting also that the fluid, in spite of its large blood content, shows no tendency to clotting. This is, indeed, quite the opposite of the prompt and firm clotting which takes place in an admixture of spinal fluid and blood of traumatic origin in performing the lumbar puncture.

In this connection, I have observed the effect of mixing in vitro, normal blood and normal spinal fluid from the same and different individuals, on the type of clot, and the clotting time. Briefly stated, the admixture will produce a firm clot in the same time as the blood alone in proportions up to 1 part by volume of whole blood to 75 parts of spinal fluid. Greater dilution of the blood will cause an increase in clotting time of the mixture, but result in a firm uniform clot up to 1 part of blood to 22 parts of spinal fluid, at which point the clotting will be delayed about fifteen minutes. A still greater dilu-

tion of the blood results in a partial clotting of the mixture up to 1 part of blood to about 35 of fluid with a corresponding but not equal lengthening of the time

The point is that even though the amount of blood in the fluid obtained in spontaneous meningeal hemorrhage be much greater than that required to clot a mixture of normal blood and fluid *in vitro* no clotting takes place

Additional spinal fluid findings will be referred to later

REPORT OF CASES

CASE 2—Mr F J white carpenter aged forty one was seen with Dr Haller at St Luke's Hospital Davenport Iowa May 17 1922

Present Illness—While shingling the roof of a house the patient was seized with a sudden excruciating pain in the head He had to be helped to the ground and was unable to walk home There was no history at that time or previously of any injury to the head He was a chronic alcoholic On entering the hospital he complained bitterly of headache was very impatient and resentful that he had had no relief

His temperature varied from 98.0 to 99.8 F pulse 50 to 78 respirations 20 to 24 RBC 5380 000 WBC 9400 Hgb 93 per cent coagulation four minutes Wassermann negative urine albumin + casts occasional hyaline Vray of head No sign of fracture Pupils Slightly dilated equal and regular direct and consensual light reflexes and to distance are prompt Eye grounds Slight clouding of the discs

Withdrawal of cerebrospinal fluid gave prompt relief of symptoms Cerebrospinal fluid Wassermann negative

SPINAL FLUID

DATE	AMOUNT	PRESSURE MILLIMETERS OF MERCURY	ERYTHRO CYTES	LEUCO CYTES	CLOT	SUPERNATANT FLUID
5/19/22	50 cc	+++	+++		0	Water color
5/20/22	25 cc	22	160 000	900	0	Amber tint
5/22/22	25 cc	17	90 000	980	0	Light amber
5/24/22	20 cc	14	10 000	150	0	Amber

The patient left the hospital at the end of ten days seeming to have fully recovered

While at work June 1 1922 he had a second attack similar in all respects to the attack two weeks previously He was again admitted to the hospital with symptoms signs and course of the illness practically parallel to his first admission Relief of symptoms followed the third lumbar puncture

SPINAL FLUID

DATE	AMOUNT	PRESSURE MILLIMETERS OF MERCURY	ERYTHRO CYTES	LEUCO CYTES	CLOT	SUPERNATANT FLUID
6/1/22	30 cc	22	210 000		0	Amber
6/3/22	25 cc				0	
6/4/22	25 cc				0	
6/5/22	20 cc				0	
6/6/22	25 cc	17	90 000	400	0	
6/7/22	25 cc				0	
6/9/22	15 cc				0	

The patient was discharged at the end of eight days and returned to work His physician told me three years later that the patient had remained well and that he continued to drink as much alcohol as ever

CASE 3—Mr D W, white, theater organist, aged forty seven, was seen at the request of Dr Haller, at the patient's home June 25, 1925

Present Illness—Returning home from work about 11 00 P M, the patient was taken with a sudden sharp severe pain in the head He remembered nothing after that until he was found in collapse on the stairway of his home Later, he had an indistinct recollection of crawling home on his hands and knees

Examination—The patient looked very ill He was irrational, stuporous, cyanotic, complaining of severe headache His neck was stiff, and head retracted A lumbar puncture yielded 40 cc of uniformly bloody fluid under increased pressure He soon became more rational, and was taken to St Luke's Hospital His temperature was 99.4, pulse 66, and respirations 22 The pupils were dilated, equal, regular, and reacted to light and distance The deep tendon reflexes were normal, there was slight but distinct general rigidity of the body He complained bitterly of headache, was restless, very active and noisy at times, and cyanotic There was improvement in his condition following repeated lumbar punctures When he became mentally clear, he could recall no cranial injury He was a total abstainer from alcoholics

Blood—Erythrocytes 4,800,000, leucocytes 10,600, hemoglobin 80 Wassermann, negative Coagulation and bleeding time four min Urine trace of albumin No sugar or acetone bodies

Spinal Fluid—Wassermann, negative

SPINAL FLUID

DATE	AMOUNT	PRESSURE MILLIMETERS OF MERCURY	ERYTHRO CYTES	LEUCO CYTES	CLOT	SUPERNATANT FLUID
6/25/25	40 cc	++	+++		0	Water color
6/26/25	25 cc	++	++		0	" "
6/30/25	65 cc	26	210,000		0	Amber
7/ 1/25	30 cc	++	++		0	" "

July 10, two weeks after admission, he was discharged, recovered Within a week after he left the hospital, he developed lobar pneumonia and died on the fifth day, but without any apparent return of head symptoms

CASE 4—Mrs H, white, housewife, aged sixty two Was seen at her home July 8, 1924

Present Illness—While sewing, the patient felt a sudden sharp pain in her head, which she described as a blinding pain She soon became restless, appeared to be in great pain, but did not lose consciousness Morphine $\frac{1}{4}$ gr hypodermically had no effect Lumbar puncture gave about 40 cc of uniformly bloody fluid under increased pressure The patient was somewhat relieved within two hours The puncture was repeated twice thereafter, with improvement in and relief of symptoms She remained at her home, and seemed to have fully recovered in ten days I have no record of further examinations in this case

CASE 5—Mr A B, white, laundry wagon driver, aged forty two, seen with Dr A H Arp, at the Moline Public Hospital, November 16, 1926

Present Illness—The patient, apparently well, drove his truck away from the laundry at 2 00 P M He was found lying on the seat of the truck about an hour later The truck had run over the curb into a vacant lot He remembered having had a severe pain in his head, and that he soon became helpless He recalled nothing more until the next day

Examination—At 6 00 P M, Nov 16, 1926, the patient looked very ill He was comatose, his breathing labored, the skin dry and cyanotic There was a general rigidity of the body He tossed from side to side and moaned His pupils were dilated, equal, round, and reacted to light The sclerae were deeply injected Examination of the reflexes was unsatisfactory on account of the rigidity

The Wassermann both on blood and spinal fluid was negative and the blood pressure was 160/95

SPINAL FLUID

DATE	AMOUNT	PRESSURE MILLIMETERS OF MERCURY	ERYTHRO CYTES	LEUCO CYTES	CLOT	SUPERNATANT FLUID
11/16/26	62 cc	+++	+++		0	Water color
11/17/26	40 cc	24	+++		0	Straw
11/17/26	36 cc	20	270 000	1 000	0	Amber
11/18/26	30 cc	22	200 000	780	0	
11/19/26	30 cc	17	220 000	800	0	
11/20/26	38 cc	10	150 000	1 200	0	
11/21/26	20 cc	16	80 000	1 300	0	
11/22/26	25 cc	+	+			

The patient left the hospital at the end of sixteen days and returned to work two weeks later. His physician reported that he is well now after two and one half years.

CASE 6—A white practical nurse aged thirty four was seen with Dr. A. E. Williams at the Mohr Public Hospital.

Present illness—The patient was left in charge of the children in a family while their mother was away from home for the afternoon. The patient seemed to be in her usual good health. When the mother of the children returned she found the patient lying on a bed unconscious. The first physician who saw her thought she had an epileptic seizure. Dr. W. saw her about an hour later and sent her to the hospital. She did not regain consciousness. During the evening and forepart of the night she was quite active at times and seemed to be in great pain. Toward morning she became deeply comatose.

Examination—At 1:00 P.M. the next day or twenty two hours after attack the patient was profoundly comatose. The face and hands were very cyanotic. It seemed that the patient was moribund and further examination was not attempted.

A lumbar puncture yielded about 50 cc of uniformly bloody fluid under greatly increased pressure. It was doubtful whether any improvement followed. She expired about five hours later as we were preparing to repeat the puncture. I had the feeling at the time that earlier spinal drainage might have changed the course of events.

CASE 7—Mr. P. A. white truck driver aged sixty. The history and clinical findings are as given by Dr. L. J. Porstmann, Davenport, Ia.

Present illness—On February 19, 1929 the patient while at work suddenly developed a severe headache, became dizzy and ill. There was no evidence then (nor at autopsy later) of any cranial injury. Dr. Porstmann saw him first on the following day. He complained of headache and dizziness which was not relieved by morphine. Later that day the patient became irrational followed by convulsive seizures and loss of consciousness. During the next three days he became very active and had to be restrained. He was given large doses of morphine. A noncooperative spirit on the part of the family prevented anything more than casual observation in this case. Counsel was not wanted and permission for a lumbar puncture positively refused. The patient expired February 24 on the sixth day of his illness. At the request of an insurance company I performed an autopsy about four hours after death before embalming had been done.

The following brief abstract of the autopsy record gives only the important findings.

There was no sign of trauma or external violence. The scalp and cranium showed no sign of injury. The skull cap averaged from 5 mm to 11 mm thick. The dura was everywhere intact, smooth, moist and normally adherent to the skull. In removing the cap the arachnoid was inadvertently cut followed by gush of bloody serous fluid (about 100 cc from the saw cut). There was still as much as 75 cc of bloody fluid between the arachnoid and pia when the cap had been completely removed. The dural sinuses contained the usual dark red firm nonadherent partially clotted blood.

The appearance of the menorrhoid was striking. It contained irregular masses of soft blood clot varying in color from light to dark red and from 3 mm to 10 mm in thickness. Several stages of organization could be easily distinguished grossly, from strings and plaques of fibrin to collections of semiliquid blood. No single source of the blood could be found. A careful search was made for vascular dilatations, aneurisms in and around the circle of Willis. The most of the blood, and the thicker more organized clots were over the upper half of the cerebrum, and about equally distributed in both hemispheres, possibly a little larger volume on the left side. The ventricles contained a bloody fluid with no sign of clotting. They were of normal size, and the communications between them were patent.

The pia mater was intact, and somewhat edematous. The brain itself was of normal consistency and contour. There were no gross changes. Serial sections showed no evidence grossly, of hemorrhage, thrombosis, embolism or tissue change anywhere in the cerebrum, cerebellum, pons, medulla peduncles, or upper cord. There was no evidence of heart, kidney, pulmonary, or liver disease.

Brief Summary of Seven Cases Reported in the Text of this Paper

INCIDENCE

CASE	SEX	AGES	OCCUPATIONS
1	Female	12	Schoolgirl
2	Male	49	Carpenter
3	Male	47	Musician
4	Female	62	Housewife
5	Male	38	Delivery man
6	Female	34	Practical nurse
7	Male	60	Delivery man

CASE	DURATION (DAYS)	OUTCOME	REMARKS
1	5 -14	Recovery	
2	5 -10 and 5 - 8	Recovery	2 attacks
3	7 -14	Recovery	
4	4 - 8	Recovery	Died of pneumonia
5	16 -30	Recovery	
6	15-	Death	Delayed drainage
7	5 -	Death	No drainage

ONSET OF SYMPTOMS

- 1 Engaged in usual occupations, previously well
- 2 No history of cranial trauma in these cases
- 3 A sudden, sharp, severe pain in the head, soon followed by symptoms of meningeal irritation. Pain usually described as excruciating
- 4 Active delirium and partial loss of consciousness

COURSE OF DISEASE (1)

- 1 Mental picture dominates with the expression of great pain, restlessness and cerebral impairment varying from delirium to profound coma
- 2 Temperatures vary from 97° to 101° F, the pulse 60 to 90, and respirations at first not much altered became slower and more labored
- 3 Eyes pupils dilated equal regular, react to light and accommodation. Spontaneous lateral nystagmus present in one case
- 4 A definite but not marked general rigidity of the body and extremities. No paralysis

5 Superficial and deep reflexes not reliably reported in this series. As a group, generally diminished and influenced by mental state.

LABORATORY FINDINGS

- 1 Blood counts (a) Erythrocytes within normal limits. Leucocytes 10 000 to 14 200. Hemoglobin 70 to 92. (b) No records of differential counts.
- 2 Coagulation and bleeding time within normal limits.
- 3 Wassermann tests all negative in 6 cases; seventh not done.
- 4 Urine examinations. Usually a trace of albumin. No sugar or acetone bodies.

CEREBROSPINAL FLUIDS

- 1 Uniformly bloody fluids under increased pressure.
- 2 Blood content and pressure diminish with successive punctures.
- 3 Leucocytes slightly increased in proportion to reds.
- 4 On standing cells settle down to make up as much as 1/20 the volume of the mixture.
- 5 Early in the attack supernatant fluid is water color. In successive withdrawals xanthochromia increases as red cells diminish and show degeneration.
- 6 There is no tendency to clotting of the fluid.

CONCLUSIONS

- 1 Seven cases of spontaneous meningeal hemorrhage are reported with reference particularly to the cerebrospinal fluid.
- 2 In five cases where drainage of the subarachnoid fluid (lumbar puncture) was instituted early and repeated there was a prompt improvement and recovery. In two cases where drainage was delayed death followed within twenty-four and thirty-six hours respectively.
- 3 A bloody, nonclotting cerebrospinal fluid under pressure is of great diagnostic importance.
- 4 As a cause of sudden and unexplained coma the possibility of spontaneous meningeal hemorrhage should not be overlooked.
- 5 Ascertaining the cause of coma particularly in emergencies, has become more and more of a laboratory problem and in some localities a member of the hospital laboratory personnel is frequently called on to direct the procedure. In fact it is in recognition of this tendency that one has presented at this meeting what might seem to be more strictly a clinical problem.

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DISCUSSION

Dr A S Giordano—I would like to add three cases to Dr Lamb's collection. It is interesting to me the ages in these cases, a boy twelve years of age and a man forty. I performed an autopsy on one out of the three and the other is only a month since the first attack. A man of thirty years of age gradually felt himself going, stopped his automobile and lost consciousness, was brought to the hospital and taken home the following day with a terrific headache. Spinal punctures were done. The fluid was under pressure, bloody and the icterus index was six on the supernatant fluid. He was kept in bed for two weeks and gradually improved. Examination revealed a definitely stiff neck. The diagnosis was meningitis partly due to the fact that they had been having considerable meningitis. The blood in the spinal fluid clinched the diagnosis. We gave him rest in bed, transfusions and injections of glucose and he was getting on very well. The nurse left him and they found him unconscious again, with similar findings in the spinal fluid. The family had another physician come in who concurred in the diagnosis. He gradually improved. A boy twelve years of age was playing bill. He stopped playing saying his head hurt him. After thirty minutes he became unconscious and came into the hospital that night. A lumbar puncture revealed tremendous hemorrhage, no clotting observed. He died about two hours after the puncture. Autopsy revealed diffuse subdural hemorrhage involving the entire brain. We could not ascertain the point of bleeding.

Dr Frederick H Lamb—With regard to Dr Giordano's question, the highest temperature observed in our series of cases was 101° F. It was mentioned that the autopsy findings were subdural hemorrhages. Does this mean literally subdural or subarachnoid?

Dr A S Giordano—Both.

Dr Frederick H Lamb (closing)—My idea of it is this. The subdural hemorrhage or hematoma is the chronic hemorrhagic pachymeningitis of Viehweg, usually a delayed manifestation or sequel of cranial trauma, whereas, spontaneous subarachnoid hemorrhage is acute, usually nontraumatic and probably due to the rupture of a small aneurism. The subdural hematoma is amenable to surgical treatment, whereas the spontaneous subarachnoid hemorrhage is not. Frequently, repeated lumbar punctures with relief of increased intracranial pressure, not only establish the diagnosis firmly, but give prompt respite of symptoms.

A STUDY ON STREPTOCOCCI IN RELATION TO PATHOGENICITY AND SUGAR FERMENTING PROPERTIES*

A PATHOLOGIC CLASSIFICATION OF STREPTOCOCCI

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OF LATE years, the important fact that streptococci do not comprise a homogeneous group has been steadily gaining credence¹. When first isolated from septic wounds streptococci were thought to play a simple pathogenic role but, from the further advance of bacteriology they have been isolated from various sources, pathogenic as well as nonpathogenic so that even now, in consideration of their role as secondary invaders knowledge of their specific pathogenicity is very confusing.

The study of streptococci really started with improvement of technique in blood culture methods, which resulted in the recognition of certain types of true streptococcic septicemias such as puerperal fever, malignant endocarditis septicemias from focal infections, etc. An opportunity was thus afforded of observing certain important biologic characteristics not common to all types, and the heterogeneity of the streptococcic group became apparent. Since then, methods have been further improved, and new ones devised that have permitted more scientific study of their biologic differences. Certain specific types such as the *Streptococcus hemolyticus*, the *Streptococcus pyogenes*, and the *Streptococcus viridans* "mitis" of endocarditis, the weak hemolytic streptococcus of scarlatina, and the *Streptococcus erysipclatis* have been differentiated. There is no doubt that persevering research will finally classify streptococci not merely according to their morphology, cultural characteristics and intricate biologic properties (factors which are not always constant), but also according to that which is the ultimate aim of bacteriology—namely their specific pathogenicity.

In the course of the last three years we have collected a series of cases of streptococcic septicemia and by applying recent biologic classifications have compiled Table I. The nomenclature used by Holman based on the fermentation of his three cardinal sugars, was used throughout.

General Remarks. The clinical classification of certain infectious diseases into acute and chronic types has a bacteriologic parallel in the blood altering properties of the streptococci that cause them. The acute conditions are caused usually by hemolytic streptococcus the chronic by *Streptococcus viridans* and by nonhemolytic streptococci.

In eleven cases of mastoiditis hemolytic streptococci were found in ten of them and *Streptococcus viridans* of the alpha prime type also weakly hemo

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lytic, was found in the other case. Four cases of acute septicemia following infected wounds were all hemolytic. *Streptococcus viridans* were found in ten patients with subacute bacterial endocarditis. The occurrence of both streptococcus hemolyticus and *Streptococcus viridans* in multiple infectious arthritis speaks also in favor of the peculiar course of this disease as regards chronicity.

TABLE I

A PATHOLOGIC CLASSIFICATION OF STREPTOCOCCI OF SEPTICEMIC ORIGIN

PATHOLOGIC CONDITION	HEMOLYTICUS					VIRIDANS				
	INFREQUENS	PROGENES	ANGINOSUS	EQUI	SUBACIDUS	FECALIS	MITIS	SALIVARIUS	IGNAVUS	TOTAL
Rheumatoid Arthritis	8	1	2		1	12	7	3	2	36
Bacterial Endocarditis							3	5	2	10
Acute Mastoiditis		6	3	1			1			11
Acute Rheumatic Fever		1					1			2
Acute Septicemia			4							4
Total	8	8	9	1	1	12	12	8	4	63

MULTIPLE INFECTIOUS ARTHRITIS

As we have dealt with this subject in previous contributions^{3, 4} and intend to deal with it more extensively in a subsequent work, we shall merely mention here certain facts of importance. These positive blood findings were obtained mostly in afebrile cases by means of a special technic depending on the inactivation of the bacteriolytic complement in the blood before it was cultured. *Streptococcus infrequens* under hemolyticus, and *Streptococcus fecalis* under viridans were the most frequently isolated. Biologically the difference between *Streptococcus infrequens* and fecalis is based on hemolysis only. Their sugar fermentations are identical. The isolation of a fair percentage of other streptococci from the blood of arthritic patients still lacks an adequate explanation, for, in our experience, with the exception of *Streptococcus fecalis* and infrequens which never failed to produce arthritis in rabbits, the only other streptococcus that occasionally produced arthritis was the mitis. *Streptococcus anginosus* and *Streptococcus pyogenes* were too virulent to cause a chronic disease. Experimentally, they invariably gave rise to an acute fatal septic condition, and, occasionally, a pyogenic joint in the case of *Streptococcus pyogenes*. We were never successful in producing arthritis with *Streptococcus salivarius*, subacidus, and ignavus. Their presence, therefore, in the blood of arthritic patients in all probability has no important bearing on the disease.

ACUTE RHEUMATIC FEVER

Our list in this category is very meager. In fact, we had only one true case whose blood culture gave *Streptococcus mitis*, raffinose positive and mulin negative. The other case with the *Streptococcus pyogenes* finding proved to be a pyogenic, post-traumatic knee-joint infection, confirmed also by the post-operative findings.

TABLE II
A CLINICAL AND BACTERIOLOGIC ANALYSIS OF TEN CASES OF BACTERIAL ENDOCARDITIS

BACTERIOLOGIC DATA										CLINICAL DATA										ELECTROCARDIOGRAPHIC RECORD		DESCRIPTION	OUTCOME	
SERIAL NUMBER	NAME	TYPE OF ORGANISM	NUMBER OF COLONIES PER PLATE	SUGAR REACTION		HOLMAN CLASSIFICATION	RHEUMATIC HISTORY	1	2	3	4	5	6	7	8	9	10	P	QRS					T
				MANNITE	SALICIN																			
1	F	St Var	100	-	-	+	Salivarius	+	+	+	+	+	-	+	9	70	M	-	+	+	Inverted T	LVP Died		
7	H	"	100	-	-	+	"	+	+	+	+	+	-	+	-	-	AM	-	+	+	Inverted T	LVP "		
10	W	"	100	-	-	+	"	+	+	+	+	+	-	+	13	81	TAM	-	+	+	Inverted T	LVP "		
29	M	"	50	-	-	+	"	+	+	+	+	+	-	+	11	84	M	-	+	+	Inverted T	LVP "		
30	S	"	100	-	-	+	"	+	+	+	+	+	-	+	47	78	M	-	+	+	Inverted T	LVP "		
5	L	"	100	-	-	+	Ignavus	-	+	+	-	+	-	+	21	61	M	-	-	-	No record	"		
11	W	"	100	-	-	+	"	-	+	+	-	+	-	+	10	40	M	+	+	+	Peaked Wide A	Recoverel		
9	E	"	30	+	+	+	Mitis	-	+	+	-	+	-	+	10	40	M	+	+	+	Auric Hypert	Died		
6	R	"	40	+	+	+	"	-	+	+	-	+	-	+	10	40	M	+	+	+				
3	L	"	100	+	+	+	"	-	+	+	-	+	-	+	10	40	M	+	+	+				
Historical data missing																								
Historical data missing																								

NOTE—The plus sign indicates the presence of the particular clinical condition. Under sugar reactions it indicates fermentation. The leucocyte count gives the figures in thousands. In Column 10, M indicates mitral, A indicates aortic, and TAM indicates mitral and aortic. LVP stands for left ventricular preponderance and A for auricular hypertrophy.

BACTERIAL ENDOCARDITIS

Comments In ten cases of typical bacterial endocarditis we found three different types of streptococci. We are thus confronted with the dilemma of either giving up the attempt at a pathologic classification of streptococci based on certain biologic characteristics, or of demonstrating that all bacterial endocarditides are not the same.

To accept the latter hypothesis as correct, it is not sufficient to show the existence of certain symptomatic variations in the clinical course of individual endocarditides, but it is necessary to prove that such variations occur repeatedly in a proportion of cases sufficient to constitute a group. Furthermore, given that the symptomatology of endocarditis warrants such a grouping, we still have to find whether this has a bearing on the specific etiologic factor.

We entered very carefully into an analytic study of the symptomatology and clinical data of the above ten cases in an attempt to prove to our own satisfaction whether or not our arbitrary biologic classification of the above streptococci had a clinical foundation. Omitting symptoms and signs common to all three groups, we recorded in Table II such data as were conspicuously dissimilar.

The differences were classed under the following six heads:

Columns 1, 2, 3 Previous rheumatic history, positive focal findings, rheumatic pains (other than directly attributable to the cardiac condition)

Columns 4, 5 Occurrence of petechiae in mucous membranes and skin

Columns 6, 7 Type of fever (sustained, septic)

Columns 8, 9 Leucocyte count and differential

Column 10 Cardiac signs by physical examination

Columns 11, 12, 13 Electrocardiographic evidences of the extent of cardiac involvement

In the bacteriologic group of *Streptococcus salivarius* we note the existence of a rheumatic history, the persistence of active infected foci and joint pains, the generalized distribution of petechiae both in mucous membranes and extensively in the skin, the septic type of temperature, at times fluctuating between 104° F. and 98° F., the relative leucopenia (the table gives the average of 10 or 15 counts in each case), with a high percentage of segmented forms, the complex endocardial involvement, double mitral in Cases 1, 29 and 30, double mitral and aortic in Case 7, and double mitral, aortic, and tricuspid involvement with partial bundle block, right, in Case 10. The electrocardiograms disclosed in addition to the (left) ventricular preponderance, myocardial involvement as evidenced in the inversion of the T-wave in all, and notching of the S in Cases 10 and 29. No signs of auricular abnormalities were detected.

In contrast to the group of *Streptococcus salivarius* which proved to be very rich in symptomatology, we take the *Streptococcus ignavus* group where the absence of most of the above symptoms is conspicuous, viz:

The absence of rheumatic history and its concomitant signs

The absence of petechiae in skin and mucous membranes with the exception of a suspicious spot in the conjunctiva of Case 5

The sustained course of temperature unlike the septic fever of *Streptococcus salivarius*

The marked leucocytosis with a low percentage of segmented forms, a marked contrast to the hematology of *Streptococcus salivarius*

The involvement of the mitral valve alone

The sparing of myocardium with an auricular hypertrophy as evidenced in the P waves of both cases

We cannot ignore such a marked contrast irrespective of the small number of cases that comprise our groups. Furthermore, the unfailing coincidence of the characteristic group symptoms under our attempted bacteriologic classification shows the importance of such concept

The group of *Streptococcus mitis* takes an intermediate position between the *Streptococcus salivarius* and *Streptococcus ignavus*

A rheumatic history and active focal signs may or may not be found

Petechiae are limited to the mucous membranes (conjunctiva in Case 9)

The type of fever is rather of the sustained type

The leucocyte count is around normal with a marked polymucleosis

The mitral valves are usually involved and the myocardium may undergo pathologic changes

It is unfortunate that our findings could not also be substantiated by necropsy records

MASTOIDITIS

In our group of eleven cases of mastoiditis *Streptococcus pyogenes* occurred seven times *Streptococcus anginosus* twice and *Streptococcus mitis* and equi each once. Here, again we meet the same difficulty of accounting for four types of streptococci in the same disease. By a careful analytical study of the clinical data we were able nevertheless to differentiate at least two different types, the pyogenes and the nonpyogenes. The data are given in Table III

Comments. The eleven cases of mastoiditis in respect to their bacteriologic and clinical features fall into two distinct categories

First, the *Streptococcus pyogenes* was characterized by metastatic abscesses in remote areas, the site of which determined the final outcome of the particular case. When the brain or meninges was involved, it proved 100 per cent fatal (Cases 4, 13, 28). Recovery, however, was the general rule with soft tissue involvement as such locations were usually accessible to surgery (Cases 14, 27). Occasionally the course of the disease was so severe and toxic that death supervened in a short time thereby offering little chance for a metastatic abscess (Case 22).

Second, all other streptococci groups combined, which were characterized by the absence of pyogenic metastases, except complications occurring in the immediate neighborhood of the mastoid such as sinus and perisinus involvement. It was significant to note that there was no fatality in this group.

Neither the severity of symptoms at the onset and during the subsequent course of the disease, nor the type of fever were pathognomonic of the kind of streptococcus isolated. Symptoms as such were equally partaken of by all

TABLE III
A CLINICAL AND BACTERIOLOGIC ANALYSIS OF ELEVEN CASES OF ACUTE MASTOIDITIS
ACCOMPANIED BY SEPTICEMIA

SERIAL NUMBER	NAME	LABORATORY DATA				CLINICAL DATA				COMPLICATIONS	OUTCOME	
		TYPE OF ORGANISM	NUMBER OF COLONIES	SUGARS FER- MENTED		HOLMAN CLASSIFI- CATION	LEUCOCYTE COUNT	SEGMENTED FORMS %	POSTHOPITATIVE DIAGNOSIS			
				MANNITE	SALICIN							LACTOSE
1	P	Streptococcus Hemolyticus	50	-	+	+	Pyogenes	22	91	Acute suppurative sclerosed	Brain abscess	Died
13	P	"		-	+	+	"	26	73	Operated outside	Meningitis	Recovery
14	K	"		-	+	+	"	16	71	Acute suppurative granulating	Subdural abscess	Died
25	A	"	50	-	+	+	"	19	84	Chronic purulent necrotic	Gluteal abscess	Died
22	R	"	18	-	+	+	"	-	-	Acute fulminating	Died in 2 days	Died
27	S	"	50	-	+	+	"	11	90	Acute hemorrhagic necrotic	Femoral abscess	Recovery
28	A	"		-	+	+	"	15	70	Chronic purulent necrotic	Meningitis	Died
12	B	"	5	-	-	+	Anginosus	10	78	Recovery without operation	None	Recovery
24	A	"	25	-	+	+	"	11	80	Chronic sclerotic granulating	Sinus phlebitis	Recovery
17	G	"	3	-	+	+	Equi	8	60	Acute suppurative	Sinus thrombosis	Recovery
8	B	Streptococcus Viridans	30	-	+	+	Mitis	8	70	Acute hemorrhagic necrotic	None	Recovery

Note—The leucocyte count gives the figures in thousands

PART IV
A CLINICAL AND BACTERIOLOGIC ANALYSIS OF FOUR CASES OF ACUTE SEPTICEMIA WITH NO TENDENCY TO LOCALIZATION

SERIAL NUMBER	NAME	LABORATORY DATA					CLINICAL DATA					OUTCOME
		TYPE OF ORGANISM	NUMBER OF COLONY	SOURS PER CUBIC CENTIMETER			ROUMAN CLASSIFI- CATION	LEUCOCYTE COUNT	FOUNDED FORMS %	CLINICAL DIAGNOSIS	PRIMARY SOURCE OF INFECTION	
				MANNITE	SALICIN	LACTOSE						
10	S. M.	Streptococcus Hemolyticus	3	-	-	+	Anginosus	11	75	Interperal sepsis	Unknown	Recovered
11	S.	"	20	-	-	+	"	10	90	Chronic cardiac arteriosclerosis	Phlebitis	Died
12	S.	"	3	-	-	+	"	12	81	Erysipelas	Paronychia	Died
13	S.	"	3	-	-	+	"	13	80	Gangrene of finger	Operative infection	Recovered

streptococci Severe septic fevers, though common with *Streptococcus pyogenes*, were not at all uncommon with such other streptococci as occur in mastoiditis Of clinical data the hematologic picture was all that showed certain group characteristics In the *Streptococcus pyogenes* group there was a marked leucocytosis, with a relative increase of the segmented forms, while the nonpyogenes group gave either a normal count or a relative leucopenia (Cases 8, 12, 17, 24)

Therefore, depending on the leucocyte count alone, we venture to make the following statement relative to prognosticating the outcome of a mastoid condition

1 If the leucocyte count is above 10,000, the involvement is probably due to *Streptococcus pyogenes*, carrying a high percentage of mortality, the latter depending on the particular localization of the metastatic abscess

2 If around 10,000, it is usually caused by *Streptococcus anginosus*, and

3 If below 10,000 *Streptococcus equi* or *Streptococcus viridans* mitis, both latter groups carrying a very low mortality

UNCLASSIFIED SEPTICEMIAS

This group of acute infections is generally called cryptogenic septicemia because the activity of the original focus being not conspicuous or having ceased to be active, leaves small clue to its origin By a careful study of the history, however, one can often trace the source It is usually a minor accidental infection of the extremities (Cases 16, 19, and probably 21), or of the mucous membranes, especially the nasal accessory sinuses In Case 26 the genital tract was suspected

In view of the acute course of the disease in this group it was natural to suspect a hemolytic microorganism as the cause We were impressed by the detection in the blood of these cases of a single type of streptococcus, the *anginosus*, with only slight variation in its raffinose fermenting property

As stated above, we have detected this same type of microorganism in a small percentage of cases of acute catarrhal mastoiditis If, then, *Streptococcus anginosus* had a specific selectivity for the mastoid, the absence of mastoid symptoms in the cases under discussion could be explained only by the remoteness of a focus to the mastoid and the relative shortness of the disease, although we had two cases with recovery where such a localization would naturally be expected Judging from the blood reaction, we presume that in both instances we were dealing with the same type of microorganism The leucocyte count in mastoids caused by *Streptococcus anginosus* varied between 10,000 and 11,000 with a differential of 80 per cent segmented and 20 per cent monocytes The average count in the nonmastoid septicemias was 11,500, with a differential of 85 per cent

CONCLUSIONS

The differentiation of streptococci into hemolytic and viridans is of sufficient clinical value to enable us to divide infectious conditions into acute (usually caused by the hemolytic) and the subacute or chronic (caused by the viridans)

By the introduction of their sugar fermenting characteristics we take a further step in their pathologic classification

In addition to the already known specific types (*Streptococcus scarlatinae*, *Streptococcus viridans*) we feel justified in also differentiating the following

Streptococcus infrequens and *Streptococcus fecalis* in subacute and chronic rheumatoid arthritides

Streptococcus salivarius mitis and *ignavus* in subacute and chronic bacterial endocarditides

Streptococcus pyogenes anginosus and *equi* in acute mastoiditis

Streptococcus anginosus in cryptogenetic (accidental) septicemias

As all streptococci partake to some degree of the general group characteristics we would naturally expect some overriding in the symptomatology of the class of infections to which they give rise. Nevertheless depending on their individual characteristics, they further subdivide known pathologic conditions into clinical subgroups. We thus have a *salivarius* endocarditis with myocardial as well as endocardial involvement extensive petechial rash on the skin and mucous membranes and a relatively normal white cell count and differential. In contrast to the *salivarius* we have another type, the *ignavus* endocarditis running a less severe course, not involving the myocardium with no petechiae on the skin and with a markedly high leucocytosis. In the case of mastoiditis we distinguish a pyogenic form apparently invariably caused by *Streptococcus pyogenes* and characterized by suppuration of the mastoid cells with metastatic abscesses either in the brain, meninges, or the soft tissues coupled with a high leucocytosis. Mortality in this group is very high. The other types (*anginosus*, *equi*, and *mitis*) are not strictly pyogenic (with the possible exception of *equi*), but give rise to an acute catarrhal, hemorrhagic or necrotic inflammation with absence of metastatic abscesses and a relatively low leucocyte count.

Although the above generalizations should be taken reservedly, due to the small number of cases observed still we consider them worth mentioning as the possible start of a working basis for the ultimate pathologic classification of streptococci.

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A DETERMINATION OF THE EFFECTIVENESS OF ACIDOPHILUS CULTURE IN FUNCTIONAL DISTURBANCES OF THE COLON*

CLINICAL AND LABORATORY RESULTS IN A SERIES OF DISPENSARY PATIENTS

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THIS study extends an investigation reported by Lynch¹ in 1928, in which a comparison was made of the implantability of the X and Y strains of *Lactobacillus acidophilus* in a series of institutionalized epileptics. The present observations were carried out in a group of unselected gastrointestinal out-patients. Such a group partakes of many of the features of an office practice, and it was hoped that the findings would give some indication of what may be expected under conditions of only partial control of the patient's habits and diet. For despite the numerous excellent laboratory and clinical studies to be found in the literature, led by those of Cheplin and Rettger^{2, 3} the application of the advocated therapeutic methods to routine practice demands further study.

The historic data of the problem of influencing for good the bacterial content of the bowel are so completely summarized in the monograph of Rettger and Cheplin that they will not be repeated. A complete bibliography to 1921 is given by those authors and is brought to 1927 by Cheplin³ and Cruickshank⁴. The recent contribution of Sagastume and Solari⁵ gives evidence that the acid gastric juice destroys the *L. bulgaricus*, probably accounting for Rettger and Cheplin's finding, confirmed by others, that the *bulgaricus* organism cannot be implanted. On the other hand, *L. acidophilus* was not appreciably affected, according to the investigators. No further reports on *acidophilus* therapy were found in the literature of the past eighteen months.

Granting that *L. acidophilus* can be made to supplant the normal proteolytic group of intestinal organisms, and granting that this is of great clinical benefit in many refractive cases of constipation, colitis, or "indigestion," as previous studies have indicated, there remains the question of the most practicable method of administration. Large daily quantities of viable organisms, reinforced with enormous amounts of lactose or dextrin, have often been proved to accomplish implantation.

The present study comprises observations on two series of patients. Our object in the first series, was to determine, if possible, whether a nominal number of viable organisms administered in broth culture rather than milk,

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over several weeks, either alone, or with small amounts of lactose, could bring about a modification of the fecal flora with alleviation of symptoms

In the second series of patients the X and Y strains were compared for implantability and clinical effect

METHOD

The first group of 18 patients, 15 of whom continued until the studies were completed was used to compare the results obtained by feeding moderate daily amounts of lactose alone of broth culture of Y acidophilus organisms alone (50-75 billion organisms) and of combined lactose and culture, each over a period of eight weeks. Four members of the group were used as controls, and received no medication. 4 received the lactose, 3 the culture and 4 the culture reinforced with lactose. The diagnoses determined beforehand in the routine workup of the clinic, included constipation, chronic colitis, cholecystitis, pylorospasm and marginal ulcer after gastroenterostomy. Selection was made only on the basis of willingness and ability to cooperate throughout the extended period. The possible therapeutic results were explained to the patients individually, their promise to cooperate secured and all medication except laxatives as necessary stopped.

The technique of study of the specimens was similar to that described by Lynch,¹ modified in minor details. Each patient was observed for three weeks reporting his daily habits and symptoms, and type of stool. Likewise three weekly stool specimens were examined grossly by Gram stain and by culture. Thus, with the patient's tabulated report a level of habits and of the normal proportions of bacterial flora was established.

At the end of three weeks administration of culture was begun in 11 of the 15 patients of the group the remainder acting as controls. Each patient was given weekly a supply of lactose, fresh broth culture or both culture and lactose, sufficient to last until his next visit. He received also a container for the fresh stool specimen and a mimeographed card on which to keep daily record of the number and type of stools, laxatives or enemas, change in symptoms, etc. The latter were checked and noted from time to time by one of us as the patient returned for his week's supplies. It is realized that the inability to control the patient in his home opens the clinical interpretation of his report to error and inaccuracy, and it can merely be stated that there was every reason to believe that the cooperative patients who completed the course followed instructions consistently.

The weekly specimens were noted as to color, consistency, age and odor. A small portion of the mixed specimen was then emulsified in sterile distilled water by an electric stirring machine. A portion of the emulsion next was diluted to an opacity of 10-12 on the McFarland nephelometer scale² and two pour plates of casein digest agar inoculated with 1 milliliter and 1 ten milliliter of a cubic centimeter of the emulsion respectively. The plates were examined after forty-eight hours' incubation with a No. 10 ocular and 25 mm objective for acidophilus colonies. Thin films of the original emulsion were gram stained by the modification of Kopeloff and Beerman,³ and the proportions of gram positive and gram negative bacilli recorded by counting 100 or 200 organisms in various fields.

RESULTS

Bacteriologic Findings—The gram stain counts, made weekly, showed in Series 1 a consistent rise in the gram-positive bacilli only among the patients receiving both culture and lactose. The average increase was 10 per cent, over the eight-week period. With culture or lactose alone, as well as with the controls the fluctuation was insignificant.

Weekly colony-counts on the agar pour-plates revealed a transient implantation in only one patient, the averages for the others ranging from an occasional acidophilus colony to 7 per cent. Two individuals showed no acidophilus colonies in any of the cultured specimens, thereby resembling the controls. Furthermore, our present laboratory results serve to confirm the statement made before that identification of the Y acidophilus colonies on culture plates is extremely difficult, and that the effectiveness of the medium is a most variable factor. The figures obtained, we feel, give inadequate record of the viable acidophilus organisms in the specimen. In short, it is again stated that the present methods of plate culture, so far as the Y strain of L acidophilus is concerned, are unsatisfactory. The gram-staining, however, was found satisfactory as an indication of alteration from the proteolytic gram negative group to the acidophilus gram-positive group. The evidence, of course that such gram-positive organisms are L acidophilus is merely presumptive.

In general, the laboratory results were disappointing. The viability of the cultures was assumed by control plating, the strains had been isolated from feces so as to assure their implantability in the human intestine. Yet with fairly large dosage, only that group using lactose as fortifying agent showed any appreciable increase in gram-positive organisms.

Clinical Findings—From a clinical standpoint the results were more encouraging. All constipated patients agreed that the stools were a little softer, less foul, and in some instances, laxatives were less frequently used. One patient with a long standing colitis, reported consistent improvement in the character of evacuations. No one was made worse. No patient developed diarrhea from the three tablespoons of lactose. The broth culture was not a disagreeable dose to take, and caused no gastrointestinal symptoms.

SECOND SERIES

The above group received only the Y strain of organisms. A second group of patients was therefore assembled in which a comparison of the implantability of the X and Y strains was attempted. Three tablespoons of lactose daily were prescribed for all of this group except the controls. Here the conditions under which the two cultures were given were made as near alike as possible. The strains were new ones kindly furnished us by Dr. Harry A. Cheplin of the H. K. Mulford Laboratories. Fresh cultures were made each week as before and a week's supply of culture and lactose was furnished each patient when he presented the specimen for examination with his daily record of defecation, laxatives, etc.

Two controls, and five patients each of the X and Y group began the course. One patient receiving X organisms and one receiving the Y were

forced to discontinue because of intercurrent illness. Four in each classification, however, continued for the five weeks of study. Two preliminary examinations were made before medication was begun. The technic described above was used.

RESULTS

Although the patients resembled those of the previous group in that they formed a cross section of the clinic only one failed to show a rise in the average of gram positive flora. This seemed to confirm the desirability of the fortifying lactose. With the λ organism the average increase was 15 per cent in the λ group 6 per cent. Again the plate cultures of diluted specimens proved relatively unsatisfactory as an index of viable acidophilus organisms. The results on dextrose whey agar were compared with peptone tomato juice agar and the colony counts were equally unsatisfactory. Two members in each group, however, consistently showed appreciable increases in acidophilus colonies.

The results would indicate also that the effectiveness of the γ strain is less apparent. Clinical improvement was slight and the gram stain figures less altered. The small number of patients observed, however, precludes any generalization.

Patient C Z in the λ group obtained a true implantation. He had been taking milk regularly in his daily diet because of a fairly recent gastroenterostomy for peptic ulcer. The beneficial effects of large quantities of sweet milk in this type of treatment are obvious and a further study is contemplated using milk as a staple of diet in order to obtain more thorough implantation in such ambulant patients.

An impression gained from previous experience in prescribing acidophilus milk or broth culture, namely that constipation, flatulence or mild colitis symptoms may be markedly benefited without much objective bacteriologic change, was borne out by the improvement described by the patients although implantation was obviously not achieved. The single colitis patient in this group obtained considerable relief, and has continued the regime.

SUMMARY

Two groups of ambulant gastrointestinal clinic patients were studied over eight and five week periods respectively to determine:

First, the relative effectiveness of lactose, of broth culture of *L. acidophilus* and of the combined culture and lactose in transforming the fecal flora and ameliorating common symptoms of intestinal stasis or irritation.

Second, the relative implantability of the λ and γ strains of *L. acidophilus*.

Observation of the first series indicates that lactose alone in daily dosage of 20 gm., or of the culture alone in dosage of 50 to 75 billion organisms, is in general not sufficient to raise appreciably the proportion of gram positive fecal organisms. Combining the culture and lactose brought about a partial change in the gram stain picture.

Clinical improvement was described in several instances, despite insignificant bacteriologic change in the weekly specimen examined.

Analysis of the results in the second series of patients indicates that while there is little difference in the implantability of the X and Y strains, the present figures tend to favor the X organisms, i.e., the particular small group studied showed more tangible clinical benefit and concomitant bacteriologic result.

The configuration of the X colonies in plate culture, furthermore, makes them more easily distinguished from the colon or enterococcus colonies. To determine an accurate count of Y colonies by the present culture methods was found a difficult and unprofitable task, in the absence of massive implantation.

The most striking results were obtained in a patient (C Z) who continued a previously imposed partial milk diet. The ease with which the acidophilus organisms became implanted suggested further that in investigation toward the most practicable method of securing implantations in an office or dispensary practice, a study be made of the effect of a partial sweet milk diet. The disadvantages of acidophilus milk, of reinforcing of the agar or broth cultures with large doses of lactose may be overcome by such a régime. It is hoped to extend this phase of the study further.

Finally, it should be recorded that in our experience, dextrose broth affords a palatable and efficient vehicle for supplying viable *L. acidophilus* in weekly quantities. The culture retains its potency for about ten days if kept in the cold.

CONCLUSIONS

1 *Acidophilus* culture alone was found in the present study not to be effective in modifying the fecal flora.

2 Lactose alone in an easily tolerated dose was not found effective.

3 The culture should, we feel, be reinforced by daily doses of lactose up to toleration, doses of 20 gm of the sugar daily having proved relatively ineffective.

4 Close observation of two groups of patients receiving the acidophilus organism, indicated, however, that frequently symptoms originating in the colon may be markedly ameliorated without laboratory evidence of successful implantation.

5 The X and Y strains, being variants of the same acidophilus organism, were again found almost equally useful. The advantage lay with the X organism chiefly because of ease of identification in plate culture. Its colonies were noted, as reported by others, often to revert to the Y type, however.

6 For the above reasons, and because of the complicated technique of culture, we feel that in routine practice the gram-staining of a film of emulsified feces is sufficient to obtain an indication of implantation. This should be done frequently, however in every case possible, for implantation is not easy or simple of accomplishment. If clinical results are not forthcoming, the need of drastic upward modification of the dosage of sugar or of both sugar and culture may be indicated by the microscopic picture.

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NOTE ON THE SERUM PROTEIN CONCENTRATION IN A CASE OF MULTIPLE MYELOMA OF THE PLASMA CELL TYPE*

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IN MARCH, 1928, Perlzweig, Delhue and Geschickte¹ reported results of protein studies upon the blood serum of a patient suffering from multiple myeloma of the plasma cell type. Using the macro Kjeldahl technique and the method of Howe,² they found a marked increase in the total protein with the increase almost wholly confined to the globulin fraction. Albumin was somewhat low. The clot which formed after the blood was drawn retracted but slightly and difficulty was found in obtaining enough serum for analysis. They gave a brief review of hyperproteinemia figures in the literature and showed that their findings were hardly approached by any others reported. Further evidence of the unusual nature of their results is shown by the figures given in a more recent article by Stalling and Winands.³ Among 481 determinations on 241 patients suffering from many different diseases there was none which showed such a high value for total protein as did this case of Perlzweig's and only one in which a value even approaching his figures was noted. No globulin concentration approximating those results was recorded. In a review of the literature for other determinations in such conditions they found only two papers bearing on the question. Jacobson⁴ isolated and weighed the Bence-Jones protein from the serum of a patient with this compound in the urine, and Rowe⁵ in a case of "myeloma of the spinal cord" with the same abnormal constituent in the urine found a normal total protein value and normal concentrations of globulin and albumin in blood serum. A further search of the literature has failed to reveal other reports upon similar material.

Recently an opportunity for studying a case of multiple myeloma of the plasma cell type presented itself. The clinical details of the case have been described elsewhere.⁶ Diagnosis was made from x-ray findings, from an examination of the type of cells in the circulating blood, from a microscopic study of a section of bone removed at biopsy, and from the repeated demonstration of Bence-Jones protein in the urine. The presence of relatively large amounts of this substance—0.9 volume per cent and 13 gm. in twenty-four hours in one specimen—and the almost complete absence of other forms of urine protein seem to be the only marked differences between this case and the one reported by Perlzweig and his coworkers.

When blood was taken for analysis, there was no unusual behavior of the clot, and serum for analysis was easily obtained. Determinations of protein in this material and in plasma were carried out by the micromodification of Howe's method described by Hawk and Bergem.⁷ The results are given in the table. Those recorded under the date of November 8 are the averages of satisfactory duplicates. Upon the other specimen only a single determination of each fraction was made. The second specimen was obtained five days after the biopsy. The chief difference between the two analyses is a decrease in the total protein concentration brought about mainly by a lowering of the

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globulin fraction. The unusually low globulin value recorded is hard to explain. In the method used this figure is calculated from the difference between actual determinations of the total protein and albumin and therefore is liable to a rather large error, but not only was this result based upon duplicate determinations, but the plasma and serum analyses checked each other in a satisfactory manner.

The results as compared with those of Perlzweig and his coworkers, show comparable high values for fibrinogen, normal rather than low values for albumin and normal to low instead of high figures for total protein and globulin. These last differences are much too large to be attributed to technical errors or to be explained by differences in the methods used. It seems not unlikely that the excretion of rather large amounts of Bence Jones protein rather than its possible retention in the body may explain the difference between the findings in this case and in that presented by Perlzweig. As already pointed out the chief difference between the two patients was the ease with which this compound could be isolated in our case as contrasted with the difficulty in demonstrating it in the other. Perlzweig has suggested that the hyperproteinemia reported by him is an expression of a systemic reaction to a foreign protein. Such an explanation would be entirely satisfactory for the difference between the findings as the reaction of two individuals to the same foreign protein may be entirely different.

Our figures showing normal to low values for protein and globulin in a case of multiple myeloma of the plasma cell type with Bence Jones protein in the urine are reported to show that the opposite findings cannot be considered specific for and diagnostic of this condition.

TABLE I*

	OCT 16 1928		NOV 8 1928	
	SERUM	PLASMA	SERUM	PLASMA
Total protein	7.30	8.94	5.27	6.02
Albumin	5.56	—	4.54	4.55
Globulin	1.74	—	0.73	—
Fibrinogen		8.79		0.81

Results expressed as per cent by volume

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LABORATORY METHODS

A CLOCK-TIMED GRAVITY DEVICE FOR DELIVERING SOLUTIONS AT CONSTANT RATES INTRAVENOUSLY*

By A. R. COLWELL,† M.D., CHICAGO, ILL.

WOODYATT'S¹ well known apparatus for delivering solutions intravenously at constant rates fulfills its purpose admirably for relatively short periods. Its use in prolonged experiments lasting overnight is hardly feasible, however, because it might be damaged seriously if left running for long periods unattended. The machine described herein obviates this difficulty. In principle it consists of a device which regulates automatically the rate of flow of a solution suspended several feet above the subject.

The essential working parts are represented semidiagrammatically in Fig. 1. The flow is regulated by means of two syringes, with weighted pistons, mounted vertically on a valve housing (*VH*) inside of which a tapered valve (*V*) rotates. The latter is simply a double two-way valve constructed so that every quarter turn the connections between the syringes and the inlet (*I*) and outlet (*O*) tubes are reversed. The suspended fluid enters the valve housing through the inlet tube and nipple. With the valve in the position shown the fluid fills the syringe (*S*₂) by raising the piston (*P*₂) to the top stop (*T*). After an interval the valve rotates one-quarter turn and the weighted piston, descending by force of gravity, expels the contents of the syringe through the outlet tube (*O*) into the vein. Simultaneously the inlet is connected with and fills the other syringe (*S*₁), which had been emptied with the valve in the first position.

The valve is rotated and timed by means of the mechanism shown at the right of the diagram. The motive force for the rotation is provided by a weight (*W*) suspended by a string wound on a spool which is fixed to a large gear (*LG*) in mesh with a small gear (*SG*) on the valve shaft (*VS*). The trip wheel (*TW*) at the end of the valve shaft is provided with four teeth mounted alternately at its periphery in the planes of the valve holes. In position and at right angles with this wheel is the gear (*G*) which is mounted on the minute hand shaft (*MS*) of an ordinary alarm clock mechanism (not shown). This clock gear turns constantly at a uniform rate, and at regular intervals it permits one of the trip wheel teeth to slip between two of its own, the next trip wheel tooth coming to rest on the succeeding clock gear tooth.

The details of the operation and construction of the timing mechanism are shown in the projected views at the extreme right of the diagram. In position 1 tooth A on the wheel *TW* is about to slip between two teeth on the constantly moving gear *G*. When it does so the valve turns one quarter turn and tooth B comes to rest on tooth 1 (position II). When rest on tooth 1. Likewise, when tooth C passes through slot 2, tooth D rests on tooth 2, and so on.

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Actually, therefore the administration consists of intermittent spurts. With a sixty tooth clock gear they occur every thirty seconds inasmuch as each of the sixty teeth permits two quarter turns of the valve. The frequency could be varied, of course, by the use of different clock gears. The accuracy of the clock is not altered by the friction between the gears even when twice the weight necessary to turn the valve is employed. By means of multiple pulleys and a proportionally heavier weight the apparatus can be made to operate at least twenty four hours without rewinding and even then the string can be rewound without interrupting the delivery by disengaging the spool and rotating the valve by hand while rewinding.

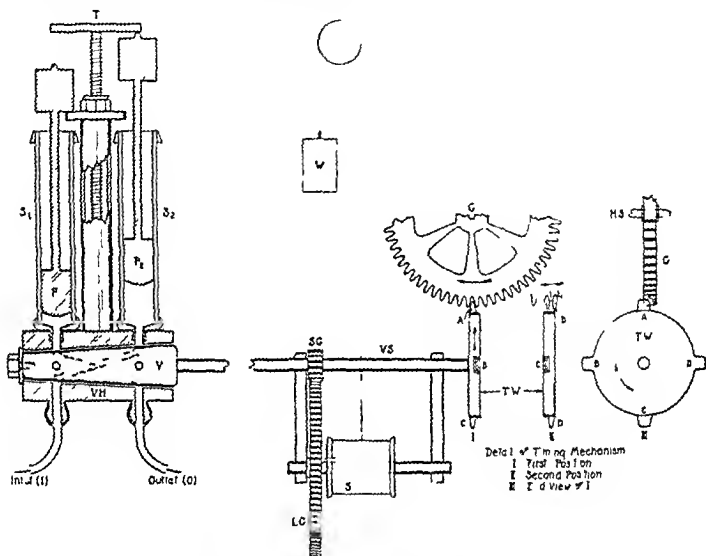


Fig. 1—Apparatus drawn semidiagrammatically

The top stop for the pistons is adjustable to different heights, the length of the stroke and therefore the delivery per hour being determined by the height of the stop. A vernier millimeter scale permits the stop to be set accurately at any desired height and hence for any predetermined delivery rate after the apparatus has been calibrated. Using 1 c.c. Record syringes and a sixty tooth gear on the clock shaft, the apparatus will permit measured administration rates within a range of 1 to 150 c.c. per hour, and by using syringes and gears of different sizes, a much wider range could be obtained if necessary. The actual delivery in any experiment may be checked easily by suspending the solution in a graduated burette. In a long series of experiments involving different rates of administration the observed delivery has never varied from that estimated by more than 1 per cent.

The lubrication of two parts deserves particular mention. The pistons operate more freely and leaking above them is negligible if they are lubricated with a light grade of sperm oil. W. F. Nye's watch oil is ideal for this purpose. The valve can be made entirely water tight by means of rubberized vaseline, prepared by melting one part of rubber tubing into about ten parts of vaseline and one part of light machine oil. Grinding and fitting the valve is the only difficult process in the construction of the apparatus. Fitting a valve which will operate freely and yet not leak requires patience and a fair degree of skill. Once suitably fitted, however, and provided with proper lubricant and motive force it does not wear appreciably. Occasional leaking or jamming is always the result of inadequate cleaning or lubrication.

Inasmuch as the injecting force is limited to about 200 cm. of water, it is true that any complete obstruction of the venous cannula will interrupt the injection. In this respect Woodvatt's apparatus is superior, especially for use with conscious animals. Yet scrupulous cleanliness of this apparatus and care to prevent clotting and twisting of the cannula is all that is required to avoid any obstruction whatsoever. In the use of anesthetized animals or perfusion preparations this feature presents no difficulties. Intraarterial administration to the normal animal is scarcely possible, but perfusions at constant rates could be performed admirably. In fact with slight changes the instrument could easily be modified to duplicate the action of the cardiac ventricle in force and frequency.

The original apparatus has given dependable service in well over a hundred experiments for an aggregate of about thirteen hundred hours. It has frequently operated through the night with no attention, the longest uninterrupted injection occupying forty-eight hours. It was made in the machine shop of Harvard Medical School under the supervision of M. F. J. Christensen, whose cooperation is acknowledged gratefully.

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METHOD OF MOUNTING PATHOLOGICAL SPECIMENS UNDER WATCH CRYSTALS AND USING A COLORED BACKGROUND FOR CONTRAST*

BY C. H. MANLOVE, M.D., PORTLAND, OREGON

THE method of mounting specimens under a watch crystal has been used for a number of years. My attention was called to this method by the work of Day.¹

The technique I wish to describe makes the mounts more durable, adds to their attractiveness for exhibition in the museum and improves the visibility of the pathology one wishes to present. It is particularly adaptable to mounting such specimens as sections of the brain, heart, kidney, and other organs. It is also suitable for mounting the smaller structures as thyroid, throat organs en masse, and uterus.

Briefly, the technique consists of mounting a specimen beneath a watch crystal (C), using a flat piece of glass as a base and securing the crystal in place by the use of some adhesive substance, filling the crystal with fluid and then utilizing as background some color which will enhance the natural color of the tissue and more clearly demonstrate the pathology desired.

I have found several factors essential to the success of this method and enumerate these as follows:

In the first place, to secure good coloring of the tissue itself the specimen is treated, as soon as possible after removal, by placing it in Kaiserling Solution No. 1, through which illuminating gas is bubbled for several hours. The carbon monoxide content of the gas is the essential ingredient as this combines with the hemoglobin to form a red carbon monoxide hemoglobin compound. It is essential that all the Kaiserling solutions be kept saturated with this gas. The specimen is then kept in a tight jar in the same Kaiserling Solution No. 1 for at least twenty-four hours. The length of time in the No. 1 solution depends on the amount of fixation required, after which it is quickly washed in water to cleanse the specimen. It may then be placed in alcohol for a short time. This is not necessary and apparently makes no marked change in the color content. The specimen is then transferred to Kaiserling Solution No. 3, which has been thoroughly saturated with illuminating gas to which I add approximately 5 per cent of Kaiserling Solution No. 1. The addition of Kaiserling Solution No. 1 prevents mold and also prevents tissue such as intestine and brain from becoming translucent.[†]

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[†]The water used in the preparation of the Kaiserling solution is practically the equivalent of distilled water.

A 22 gauge hypodermic needle is now embedded into the dam of seam filler so that it extends through to the inner part of the dam

The specimen which is to be mounted and which has been cut and prepared to fit beneath the crystal is sponged with a dry cloth to remove all excess fluid and placed inside the dam on the glass base

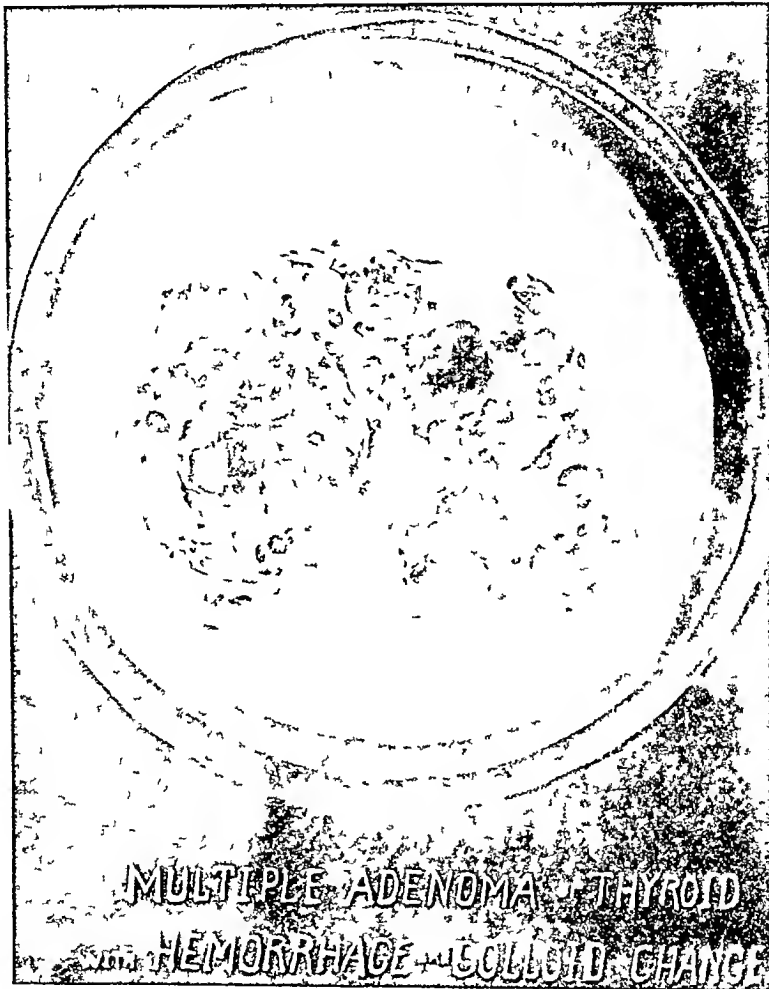


Fig 3

Gentle heat is applied to the dam of seam filler and the watch crystal to be used is pressed firmly into it. This can be successfully accomplished by applying heat to the edges of the watch crystal while continuous pressure will gradually force the crystal into the dam until the crystal comes in contact with the base, and a rim of seam filler has welled up the inner sides of the crystal for at least one-half an inch. Now allow the mount to cool, and keep enough weight on the crystal to hold it in place although there is little danger of it moving if the specimen beneath has been properly prepared. The

heat applied to the crystal should not be such as to injure the specimen. I have never had this occur even though the heat has frequently broken the crystal.

After cooling remove by means of a hot spatula or putty knife all the excess seam filler from the outside except that in immediate contact with the outside half of the ground pathway. Then clean base outside of pathway with xylol.

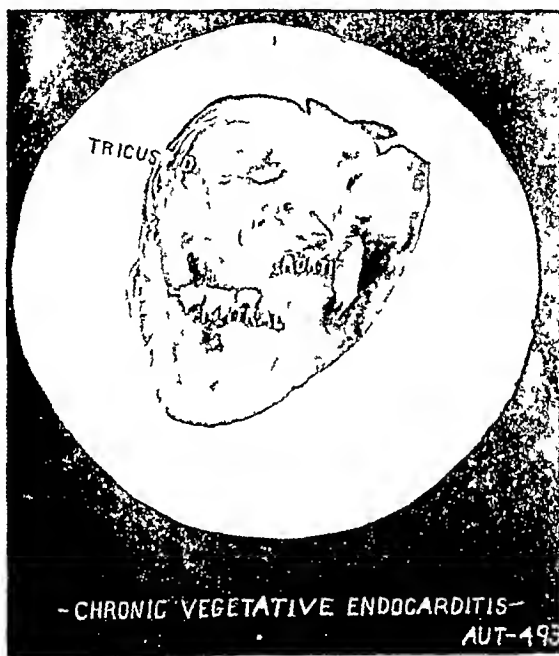


Fig 4

Now apply a rim of the seam filler to the outside edges of the watch crystal. To apply this so that a smooth even rim is formed heat the seam filler and allow to partially cool then pour slowly while holding the entire mount and rotating so that the material runs evenly around the entire circle of the crystal. If the material is at a proper temperature this may be placed so that it is smooth and fairly even. It may then be smoothed with a hot spatula.

Again allow the mount to cool and then begin to introduce the Kaiserling Solution No. 3 through the 22 gauge needle placed as mentioned. This needle

in the meantime, has acted to allow a free flow of air into and out of the chamber beneath the crystal. The fluid is introduced by means of a 10 cc syringe by alternately injecting a few cc of fluid and then withdrawing a similar amount of air. If too large an amount of fluid is forced into the chamber under pressure, it will cause separation of the crystal from the base. Continue to fill the chamber until a fairly good sized air bubble remains. At this time remove the needle by rotation and traction. The remaining bubble of air is removed by placing the specimen beneath water or under a running faucet and then by gently pressing on the crystal, force out the air and allow water to completely fill.

While the mount is still beneath water, take a match or a wooden applicator and gently tampon some of the seam filler into the opening left when the needle was removed.

There is now left the problem of completing the external rim of seam filler. The specimen should be thoroughly dried, especially where the needle opening was placed. I usually remove about 1 inch of the external rim of seam filler on each side of the needle opening and after thoroughly drying this area apply the seam filler as before. Allow it to cool and smooth with spatula. It is necessary to use much care in this last step or a leak may occur. If the seam filler is applied when too hot it will melt that which was tamponed into the needle opening. Finally smooth the external rim with gentle heat and clean the surrounding glass with xylol.

After complete cooling, apply a coat of black enamel to the external rim of seam filler and also paint the back of the glass base except for the area covered by the watch crystal.

To place the label, I find white golf ball enamel to be excellent material. It is necessary to thin it to writing consistency. For this, xylol may be used. The label may be placed with a printing pen or by use of a stencil. This enamel will dry within a week so that the glass may be dusted or washed without danger to the label. (See Figs 2, 3, and 4)

The attractiveness of the mounts and the visibility of the specimens are much improved by the use of colored backgrounds. This is accomplished by the use of colored cardboard which is placed back of the mount so as to form an outline of the specimen, giving a contrasting or enhancing color to the space within the watch crystal that is not taken up by the specimen.

I have found that orange and deep green are the most commonly suited colors. However these do not have to be in contrasting shade to the specimen, because in many I find that the orange will markedly improve a tissue with considerable red color of its own. The orange is of much help in displaying tissue with but little color. The green is of most help with tissues which have a deep, bright red. Other colors such as yellow, dark red, light green, and occasionally a bluish green will suit a small number of the specimens. These colors are selected by a process of elimination, by trying first one, then another.

The use of the color scheme with the base painted black, as de-

scribed, and altogether mounted on a black bench presents a museum display that is pleasing to look at and certainly increases the visibility of gross pathology.

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GOOD SAMARITAN HOSPITAL

A QUANTITATIVE TUBERCULIN TEST*

By JOHN E. BLAIR, PH.D., AND WALTER I. GALLAND, M.D., NEW YORK CITY

THE diagnosis of tuberculous lesions, particularly joint tuberculosis, manifestations of which may be obscure, is as a rule not clarified by the classical von Pirquet test. It is generally accepted that this diagnostic method is of little use except in the period of infancy and very early childhood, when a positive von Pirquet is presumptive evidence of an active tuberculous infection. Soon after, most individuals develop some degree of tuberculin hypersensitivity because of latent or previous infection sufficient to produce a cutaneous response to the usual scarification tuberculin test. Such reactions are as a rule of no clinical significance, and for this reason diagnosis by the aid of tuberculin in the form of the von Pirquet test has been largely abandoned as a useful procedure, except in the early years of life.

Since the von Pirquet test is purely qualitative, attempts have been made to develop a quantitative tuberculin test. These tests, which have been summarized by Hamman and Wolman of Johns Hopkins,¹ have for the most part proposed some modification of the von Pirquet technique, in which varying dilutions of tuberculin have been applied to the scarified skin, the sensitivity of the patient being subsequently estimated by the size of the wheal at the site of application of each dilution. The results in general proved to be of no more value than the ordinary von Pirquet.

The Mantoux test consists of administering OT intracutaneously in a dilution of 1:10,000. Sensitivity to the tuberculin in this dilution is considered a reaction of diagnostic value, but as no series of dilutions is used, we believe that it has no greater value than the ordinary von Pirquet. Hamman and Wolman proposed an intracutaneous quantitative test, based on the Mantoux reaction, in which they employed three intracutaneous injections of 0.05 cc of OT diluted to 1:10,000, 1:100,000, and 1:1,000,000. Smith² used a similar test, but carried the dilutions to 1:10,000,000. We have been unable to find a report of the application of either of these tests to an extensive series of cases.

Atsatt,³ working at the Children's Hospital and the Massachusetts General Hospital in Boston, described in 1927 a modification of the Mantoux intracutaneous test, which he considers to be of value in the diagnosis of bone and joint tuberculosis. The essence of his quantitative reaction consists of establishing a threshold of tuberculin sensitivity, above which threshold tuberculin allergy would warrant a presumptive diagnosis of clinically active tuberculosis, but below which threshold one might expect various degrees of tuberculin allergy to be exhibited by persons carrying latent or healed tuberculosis. Using Saranac human tuberculin, a dilution of 1:7500 was established as the threshold of reaction. The test as he describes it, consists of administering, at

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one session, intracutaneous injections of 0.1 cc of Saranac tuberculin in dilutions of 1:1000, 1:2500, 1:5000, 1:7500, and 1:10,000 together with a saline control. A cutaneous response to the 1:10,000 dilution is considered as being significant of the presence of clinical bone or joint tuberculosis, in the absence of other tuberculous manifestations. He reports a series of tests covering 211 cases, 85 of which were tuberculous upon whom this quantitative reaction was performed. Considering his cases, irrespective of age groups, 90 per cent of the nontuberculous cases gave a negative reaction, while 92 per cent of the tuberculous cases gave a positive reaction that is, positive in the dilution of 1:10,000.

Stimulated by the work of Atsatt we attempted to repeat his quantitative test on cases in the various services of the Hospital for Joint Diseases using tuberculin furnished by the New York Department of Health. Following the technique described by Atsatt, we performed the test upon 11 patients exhibiting a variety of bone and joint diseases, but obtained conflicting results. Our greatest difficulty was with cases known to be clinically nontuberculous, in some of whom we obtained florid positive tuberculin reactions with the dilutions proposed by Atsatt. However we were under the definite impression that the quantitative intracutaneous test might have usefulness. Inasmuch as we obtained florid reactions in nontuberculous cases in the 1:10,000 dilution, we thought that the tuberculin we used might be more potent than the Saranac tuberculin employed by Atsatt.

Consequently we continued our investigations but used dilutions of 1:10,000, 1:25,000, 1:50,000, 1:75,000 and 1:100,000 of the New York Department of Health tuberculin, made up in normal salt solution to which 0.5 per cent phenol was added as a preservative. Using these dilutions in a similar manner to that described, upon a series of 144 cases covering all age groups and including tuberculous and nontuberculous individuals, we concluded that a positive local reaction in dilutions of 1:10,000 and 1:25,000 was obtainable in many clinically nontuberculous cases, and that response to these dilutions had no constant diagnostic value. However, positive reactions to dilutions above 1:25,000 were subsequently proved to have considerable diagnostic significance. These results showed in addition that the Mantoux test, as performed in this city, could be misleading, since the Department of Health, using the same tuberculin, considers reaction to the 1:10,000 dilution as significant. We have therefore established the dilution of 1:25,000 as the critical threshold in the quantitative test as we perform it. In practice, the critical threshold for each lot of tuberculin used may easily be determined by a few tests on tuberculous and nontuberculous cases, and dilutions may then be prepared sufficiently high above the threshold to give significant results.

In order to explain the different thresholds proposed by Atsatt and ourselves, we prepared dilutions of Saranac tuberculin from 1:10,000 to 1:100,000 and used the Saranac and New York Department of Health tuberculins in parallel series on the same cases. We concluded that the Saranac tuberculin was distinctly less potent than the Department of Health preparation. This may account for the difference between our critical threshold and that used by Atsatt. We feel however that it is preferable to use a tuberculin admitting of high dilution in order to reduce the absolute quantity of tuberculin

protein administered. A dilution of 1 1000 of many of the tuberculins obtainable appears to be too concentrated to permit of its routine use in tuberculous subjects.

The test in the completed form has consisted of the intracutaneous injection of 0.5 to 0.1 c.c. of the diluted tuberculin upon the volar surface of the forearm. A control injection of phenol-saline solution was also made. The tuberculin was injected in dilutions of 1 10,000, 1 25,000, 1 50,000 and 1 75,000 and 1 100,000. The dilutions were kept on ice and were discarded at the end of a week to ten days, at which time they were still potent. The syringes and needles were thoroughly cleaned prior to use. The tests were read at the end of twenty-four and forty-eight hours.

The maximum positive reaction usually appears during the first twenty-four hours, but we have noted a number of cases in which the development of the reaction was delayed, and did not reach its fastigium until the end of forty-eight hours. The positive reaction consists of the formation of an indurated cutaneous nodule at the site of injection, with or without a varying amount of erythema surrounding the nodule. Occasionally a bleb forms at the summit of the nodule in the more florid reactions. We would particularly note that we consider induration as the most important criterion of reaction. We have frequently observed erythema in sensitive skins even with the phenol-saline control, and in colored patients, it is quite impossible to judge the presence of erythema. The induration is best detected by gently rubbing the finger over the volar surface of the arm at the sites of injection. A definite shotty nodular sensation is imparted. The size of the nodules obtained varies with the susceptibility of the individual, the character of the skin, the amount of fluid injected, and possibly other unknown factors. Even when small, the induration is definite and unmistakable.

In reading the results, we consider as positive, and indicative of clinically active tuberculosis, those cases which show a reaction of induration in all dilutions, except the saline control. Occasional cases have exhibited a reaction up to and including the 1 50,000 dilution, and have been negative above that. We have considered these cases as negative. We are under the impression that such reactions indicate a variation from the threshold. We feel that the use of several dilutions, well above the critical threshold, gives a sufficiently wide margin for the occasional variation, and at the same time the higher dilutions will have diagnostic value.

In the 144 cases included in this report, both tuberculous and nontuberculous adults and children are represented. We have attempted to confirm the results of the quantitative reactions whenever possible, either by guinea pig inoculation, direct smear, x-ray, or pathologic examination of tissue.

We can summarize our results as follows. Of 78 cases of all ages finally considered as tuberculous, 71, or 91 per cent, gave a positive reaction in all dilutions, and 7 cases, or 8.9 per cent, gave an entirely negative reaction. Of 66 cases finally considered to be nontuberculous, 60, or 90.9 per cent, gave a negative reaction which was of diagnostic significance, while 6, or 9 per cent, gave a positive reaction in all dilutions.

The failure to obtain a positive reaction in approximately 9 per cent of tuberculous patients does not, however, invalidate the usefulness of the pro-

cedure It is a known fact that in patients suffering from an overwhelming tuberculous infection, or in patients exhibiting but little resistance to the progress of the disease there is frequently a total absence of tuberculin allergy, and though the allergic response is negative, there are other criteria for making a positive diagnosis. Such patients are considered as having a poor prognosis. Furthermore such conditions as sepsis, intercurrent infections, and anemia may result in a lowering of tuberculin allergy. Among our tuberculous cases exhibiting negative reactions, there were 2 patients who died within a short time after we saw them, and 2 patients who are rapidly succumbing to the disease. At the time we read the reactions on these patients we stated that we considered the prognosis unfavorable. A fifth case of tuberculosis with a negative reaction was a patient who had previously received a rather protracted course of tuberculin therapy and was therefore rendered tuberculin immune to the minute doses used. This leaves but two patients to be accounted for, and we have not been able to follow them up.

In regard to the 9 per cent of positive reactions in patients the clinical lesion of which was finally considered nontuberculous all but 2 of these 7 were adults, and we are by no means certain of the absence of some undiscovered focus of active tuberculosis. It is such cases that give us the most trouble, and it is these cases which emphasize the need of utilizing every available procedure for the diagnosis of tuberculosis.

It is difficult to consider each of the 144 cases tested in detail. We will confine ourselves to a few cases which will perhaps serve to demonstrate the possible clinical value of quantitative tuberculin reactions in establishing a diagnosis.

CASE 85—L I, male aged twenty nine. Admitted to the hospital with diagnosis of tuberculosis of the knee. The patient had suffered several years with an infection of the knee which had been treated in several other hospitals, and which had been diagnosed as tuberculosis. The reaction obtained in this case was negative in all dilutions above 1:10,000 but positive in this dilution and in the von Pirquet. The result of this test was reported as negative. The patient was operated upon, the popliteal swelling was incised, and a gumma was found. The Wassermann was 4+.

CASE 94—E D, male aged fifty seven. Admitted with the diagnosis of tuberculosis of the knee. Four months before admission he had a swelling of the left hand and right knee. The patella was floating, and the motion of the knee was limited. A clinical diagnosis of tuberculosis of the right knee was made. The x-ray examination showed a moderate thickening of the apical pleura, which was reported as probably a healed tuberculosis. The quantitative tuberculin reaction was negative in all dilutions. An arthrotomy was performed, and the tissue which was removed from the knee was reported as chronic nonspecific synovitis nontuberculous.

CASE 22—A S, male, aged seventeen. Admitted to the hospital with diagnosis of epiphyseolysis. While in the hospital, he developed a pleural effusion of unknown origin. Guinea pigs inoculated with the pleural fluid were positive for tuberculosis. Previous to the development of the effusion,

the quantitative test had been performed, and it gave a positive reaction in all dilutions

CASE 13—L B, male, aged thirty Admitted to the dispensary with diagnosis of old osteomyelitis of the femur and ankylosis of the knee joint The x-ray diagnosis was old destructive arthritis of the knee, probably gonorrheal This patient was treated in the dispensary on the assumption that it was gonorrheal ankylosis of the knee The quantitative test revealed a fluid reaction in all dilutions A small area of fluctuation was detected on the medial aspect of the knee, and aspirated Guinea pig inoculation of the aspirated fluid was positive for tuberculosis

It is unnecessary to comment upon these cited cases, except to state that the use of the quantitative tuberculin reaction has in a number of instances led to the subsequent reversal of the original diagnosis

In all tuberculin work the objection has been repeatedly urged that the administration of tuberculin frequently engenders an exacerbation of the tuberculous process, and a dissemination of the infection One case in our series developed tuberculous meningitis about a week after the performance of the quantitative test We are not inclined to attribute this complication to the tuberculin administered The case in question was a child, five years of age, admitted with a diagnosis of tuberculosis of the hip, who gave an entirely negative quantitative reaction in all dilutions, and was recorded as having a poor prognosis Two days following the administration of the quantitative test, a diagnosis of miliary tuberculosis was established, but in retrospect the child had shown evidences of meningeal involvement even before the test was made We do not believe that the dissemination of tuberculosis in this child resulted from the intracutaneous injection of the extremely minute amount of tuberculin present in the dilution we used Furthermore, since this child was insensitive to tuberculin, how could the injection of dilute tuberculin lead to a dissemination of the infection? We feel that the intracutaneous use of tuberculin, even in fairly large amounts rarely results in a focal or general reaction The Mantoux test, which is quite widely used, involves the administration of 1/100,000 c c of OT The test as we perform it, uses a total of about 1/50,000 c c, which we feel is an infinitesimal dose, and is quantitatively incapable of producing any focal or general reaction, particularly as this is injected intracutaneously In addition, in our series of 144 cases, in which the skin was always carefully prepared before injecting the tuberculin dilutions, there has not been a single instance of infection or other unfavorable results referable to the administration of the quantitative test

The question may be fairly asked whether the quantitative tuberculin reaction gives any more information than the classical von Pirquet Practically all of these cases have been controlled either by the von Pirquet or by a modification of that test In forty cases in which the von Pirquet technic was used, the reaction of the latter in nontuberculous patients was comparable to that obtained with the dilutions below our critical threshold These comparative reactions would signify that frequently the von Pirquet response may be positive in cases in which the quantitative test would establish as definitely negative It has been shown that in nontuberculous individuals over ten to fifteen

years of age, positive von Pirquet or Mantoux reactions may be obtained in from 55 per cent to 89 per cent of cases, as cited by Calmette.⁴ In our small series of cases of nontuberculous individuals over ten years of age, the number of positive reactions below the critical threshold approaches von Pirquet's own figures. However, it appears to be of significance that we have obtained 91 per cent of diagnostically significant negative reactions in these nontuberculous patients.

We would conclude by stating that we consider the quantitative intracutaneous tuberculin test as a valuable accessory in the diagnosis of active tuberculosis in all age groups. We do not consider the test infallible, but we feel that its accuracy is comparable to that of many of the commonly accepted serologic and immunologic diagnostic reactions. The reaction must be considered in conjunction with all other available clinical and pathologic data. Intelligently used it will perhaps aid in establishing a final diagnosis in many obscure cases particularly in adults.

SUMMARY

A quantitative tuberculin test has been elaborated, which is based upon a similar test proposed by Atsatt but has been modified to give an apparently greater differentiation between clinically active tuberculosis and latent tubercular infection.

The test consists of intracutaneous injections of 0.1 c.c. of OT diluted with phenol saline to 1:10,000, 1:25,000, 1:50,000, 1:75,000 and 1:100,000, together with a saline control. Reactions are read after twenty-four and forty-eight hours. A positive reaction consists of the formation of an indurated cutaneous nodule, with or without erythema, at the site of injection. With the tuberculin used, a critical threshold of 1:25,000 was established, above which threshold positive reactions were considered as presumptive evidence of clinically active tuberculosis. Positive reactions below the critical threshold, with no reaction above the threshold, appear to indicate latent tubercular infection or healed lesions.

In a series of 144 cases including tuberculous and nontuberculous individuals of all age groups 91 per cent of the tuberculous cases gave a positive reaction in all dilutions while 90 per cent of the nontuberculous cases gave a diagnostically significant negative reaction.

When intelligently performed, and used in conjunction with all other available clinical and pathologic data, the test appears to have value as an accessory in the diagnosis of clinically active tuberculosis.

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APPLICATION OF THE CORPER-UYEI CULTURE METHOD IN THE ROUTINE EXAMINATION OF SPUTUM FOR TUBERCLE BACILLI*

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RECENTLY there has appeared a new method¹ of cultivation of tubercle bacilli from contaminated materials which has been claimed by Corper² to be superior to other culture methods and to the smear tests, and of equal efficiency and more advantageous than the guinea pig inoculation method³ for the laboratory diagnosis of tuberculosis. In view of the demonstrated relative uncertainty of the ordinary stained smear for the detection of tubercle bacilli in sputum² this new culture method suggested a study to determine the efficiency of the old routine smear examination as compared with sulphuric acid-crystal violet-potato medium culture method, with a view to its possible adoption as a routine procedure in the examination of sputum in the public health laboratory. Accordingly, a series of specimens of sputum during a seven-months' period was subjected to culture according to the Corper-Uyei technique in addition to the regular (carbolfuchsin stained) smear examination, and the cultures thus obtained were injected into guinea pigs to determine the pathogenicity of each strain of tubercle bacillus isolated.

METHOD

When a specimen of sputum arrived at the laboratory, it was smeared, stained, and examined. As soon as practicable a culture was made. At times it was necessary to store the sputum in the cold room as long as thirty-nine days after being delivered to the laboratory for diagnosis, before it could be cultured. This storage period, at about 4° C, did not appear to affect the viability of the tubercle bacilli, as evidenced by growth subsequently on the crystal violet-potato medium. The average period from the date of collection of the sputum from the patient until its cultivation in the laboratory was approximately seven and five-tenths days.

1 *Smear*—The routine smear examination consisted of selecting a purulent fleck of sputum (if present), smearing it uniformly on a clean glass slide, fixing it in a flame, staining by the Ziehl-Neelson carbolfuchsin-acid alcohol-methylene blue counterstain technique, and then examining the smear microscopically for acid-fast bacilli. Careful microscopic search was maintained for at least five minutes before a report of a "negative smear" was made, and the detection of a minimum of 8 acid-fast rods resembling tubercle bacilli was the requisite for a "positive smear" diagnosis. When one or more acid-fast bacilli were detected during the minimal five-minute period, the examination

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was prolonged indefinitely in an attempt to discover the number of organisms necessary for a positive diagnosis

2 Culture—The method of culture is essentially that described by Corper, with minor modifications. For convenience the sputum was transferred from the standard cubical collecting bottle to a sterile Petri dish, then smeared and cultured immediately, or placed in the cold room for later cultivation. One c.c. of unhomogenized sputum (in some instances less was available) was transferred by means of a sterile pipette into a sterile, graduated and corked 15 c.c. conical centrifuge tube and an equal quantity of 6 per cent sulphuric acid (by volume) added. A thorough mixture was obtained by beating the contents of the tube with a sterile glass rod having an enlarged roughly button shaped end. The stirring rod was gently and rapidly oscillated to and fro in the mixture for about a minute, the impingement of the button shaped end into the converging conical end of the centrifuge tube insuring a fine dispersion of the particles of sputum. In this manner the acid was allowed to permeate the sputum and exert its bactericidal effect on contaminating organisms present. Many such contaminants, found frequently in sputum, such as yeast, spores, staphylococci streptococci and *Aspergillus niger* will be eliminated if special care is taken to obtain adequate mixing of the sputum and acid at this point in the procedure. The centrifuge tube was then stoppered and incubated at 37° C for half an hour. The contents were next diluted with about 10 c.c. of sterile 0.85 per cent sodium chloride solution, mixed well by inversion of the tube several times, and centrifugalized for five minutes at a moderate rate of speed. The supernatant fluid was decanted and a generous portion of the residue planted on the slant surface of the crystal violet potato medium. Then the cotton plug, impregnated in its lower half with hot vaseline paraffin mixture was inserted into the tube and allowed to cool and harden. Finally the plugged tube was capped with tin foil and incubated at 37° C for a minimal period of ten weeks.

The medium is prepared as Corper² originally recommended 'by cutting large, clean peeled potatoes free from surface defects, into cylinders about 3 inches long and $\frac{5}{8}$ inch in diameter. The cylinders are halved [diagonally and] longitudinally and immediately soaked in 1 per cent sodium carbonate solution containing 1/75,000 or 0.0015 per cent crystal violet (the dye and sodium carbonate should be mixed just prior to use to prevent decolorizing) for from one to two hours. After this the cylinders are gently wiped off with a clean towel, and are introduced into a sterile culture tube (6 x $\frac{3}{4}$ inch size) containing 15 c.c. of 5 per cent glycerol broth, cotton plugged and are sterilized in an autoclave at 15 pounds pressure for at least thirty minutes''

3 Guinea Pig Inoculation—As a check on the pathogenicity of the acid fast bacilli obtained in each culture guinea pigs were inoculated subcutaneously with 1 c.c. of an homogeneous suspension of 2 or 3 loopfuls of the growth in 2 c.c. normal saline. The acid fastness of the organisms in the culture was determined beforehand and in the event of a sparse initial growth, transplants were made on fresh medium to insure liberal stool cultures. The inoculated animals were weighed, placed in separate cages, and at the end of six to eight weeks were sacrificed by ether anesthesia and autopsied. Anatomic tubercu-

lous involvement of the organs, especially the regional lymph nodes, spleen, liver and lungs was noted, and smears from these organs were examined for acid-fast bacilli

RESULTS AND DISCUSSION

Contaminants—The occurrence of contaminating organisms which overgrow the colonies of tubercle bacilli on the crystal violet-potato medium and which thus delay or inhibit the culture of some specimens, is an important factor in determining the practicability of the Corper method as a routine procedure in the public health laboratory. Table I shows the loss from contaminating organisms which may be expected when this culture method is introduced as a routine laboratory procedure, in the hands of anyone unpracticed in its technique, and during its development as a standard method for any particular laboratory

By chance, during the first three months (September, October, November) when the percentage of contaminants was highest (averaging 13.5 per cent), these 26 contaminated cultures contained material from 5 specimens, from which smears were positive for tubercle bacilli. The examination for the growth of the acid-fast organisms on the culture medium naturally ceased with the overwhelming overgrowth of contaminants. Therefore, a fair comparison of these 5 positive smears with the same 5 potentially positive cultures is impossible. It will be seen in Table II, however, that this early advantage of the smear method is exactly compensated for by the same number of excess positive cultures. Referring to Table I again, it is apparent that the frequency of contaminations diminishes as skill is acquired, and in this instance as factors contributing to more frequent contaminations were eliminated. Thorough mixing of the acid and sputum is essential. At first a mortar and pestle were employed for this purpose, but this apparatus was too bulky and allowed too ready exposure to air contaminants. Accordingly button-shaped glass rods and conical centrifuge tubes were adopted⁴ with a definite reduction in the percentage occurrence of extraneous organisms growing on the crystal violet-potato medium.

Contaminants in all instances in Table I appeared within the first week of cultivation. Since the tubes were not opened during this period, it is as-

TABLE I

CONTAMINATIONS IN ROUTINE CULTURES OF SPUTUMS FOR TUBERCLE BACILLI BY THE CORPER UYEI METHOD

MONTH	TOTAL NUMBER OF SPECIMENS	TOTAL NUMBER OF TUBES	CONTAMINATED CULTURES		
			NO DISCARDED	% DISCARDED	POSITIVE SMEARS
1928					
September	45	45	6	13.33	2
October	88	151	15	17.04	2
November	59	118	5	8.47	1
December	35	70	4	11.43	0
1929					
January	18	36	0	0.00	0
February	57	114	2	3.51	0
March	48	96	0	0.00	0
Totals	350	630	32	9.14	5

sumed that no invasion occurred from the air or through the vaseline paraffined tin foil topped cotton plug and that surviving contaminants were derived from the sputum or were air borne only during the handling of the materials for culture

Adequacy of Culture Compared with Smear—A comparison of the efficiency of the smear and culture methods in the detection of acid fast bacilli over the seven month observation period is recorded in Table II. It is seen that of the 318 uncontaminated specimens of sputum cultured, 38 or 11.95 per cent were "positive cultures" and only 33 or 10.37 per cent 'positive smears' a difference of 5. This difference represents an actual gain of 14.8 per cent above the 33 specimens classified as positive by the examination of the stained smear. Table III is an elaboration of Table II on the basis of uncontaminated cultures of specimens the ratio of total disagreements is 10 'positive culture negative smears' to 5 'positive smear negative cultures' a total disagreement of 4.72 per cent. Thus in the month of October there was agreement in diagnosis of 95.88 per cent in 73 uncontaminated specimens (of sputum examined for acid fast organisms by both methods) and a disagreement of 4.12 per cent or in 3 specimens. Of these, all 3 specimens grew tubercle bacilli in culture while no acid fast bacilli were detected in the smear examination. On the other hand almost the reverse was true in December when there were 2 'positive smears with negative cultures' and no "positive culture negative smears."

TABLE II

COMPARISON OF THE ACTUAL NUMBER OF POSITIVE SPUTUMS OBTAINED BY SMEAR AND BY THE CULTURE METHOD

MONTH	SPECIMENS		NUMBER POSITIVE SMEARS	PER CENT POSITIVE SMEARS	NUMBER POSITIVE CULTURES	PER CENT POSITIVE CULTURES
	TOTAL	UNCON- TAMINATED				
1928						
September	45	39	2	5.13	3	7.69
October	88	73	6	8.22	9	12.33
November	59	54	9	16.66	8	14.83
December	35	31	5	16.13	3	9.68
1929						
January	18	18	2	11.11	2	11.11
February	57	55	7	12.73	11	20.00
March	48	48	2	4.17	2	4.17
Totals	350	318	33	10.37	38	11.95

The importance of eliminating contaminants has been brought out and is herewith reemphasized. Thus the gain of 5 or 14.8 per cent positive cultures (see Table III) representing the advantage of the sulphuric acid crystal violet potato medium method over the smear stained by the Ziehl-Neelson technique is neutralized by the loss of 5 cultures by contaminants which were made from sputum showing acid fast bacilli in the smear.

It is apparent that each method has both advantages and disadvantages. An increase in the number of positive diagnoses of tubercle bacilli in sputum specimens can be made by the employment of the culture method as a supplement to the examination of the stained smear in cases in which no acid fast organisms are found in the smear.

TABLE III

VARIATIONS IN AGREEMENT OF DIAGNOSES BY SMEAR AND BY CULTURE (ON THE BASIS OF UNCONTAMINATED CULTURES)

MONTH	SPECIMENS		AGREEMENTS				DISAGREEMENTS			
	TOTAL	UNCON- TAMIN- ATED ON CULTURE	NEG CULT NEG SMEAR	POS CULT POS SMEAR	TOTAL	PER CENT	POS CULT NEG SMEAR	NEG CULT POS SMEAR	TOTAL	PER CENT
1928										
September	45	39	36	2	38	97.44	1	0	1	2.56
October	88	73	64	6	70	95.88	3	0	3	4.12
November	59	54	43	6	49	90.74	2	3	5	9.26
December	35	31	26	3	29	93.55	0	2	2	6.45
1929										
January	18	18	16	2	18	100.00	0	0	0	0.00
February	57	55	44	7	51	92.74	4	0	4	7.26
March	48	48	46	2	48	100.00	0	0	0	0.00
Totals	350	318	275	28	303	95.28	10	5	15	4.72

In Table III it may be noted that in several instances stained smears of the sputum revealed acid-fast bacilli whereas in the subsequent culture on the crystal violet-potato medium no growth of tubercle bacilli was obtained. The explanation for the failure of these specimens to grow colonies of the organisms is not clear. Malkan⁷ notes that a positive sputum may give growth on one occasion and at other times may not and admits his inability to offer an explanation. For the same reason, that he observes that ordinary cultures of tubercle bacilli when subjected to acid-digestion frequently grow out, and previously positive sputums do not result in growth by culture, it is possible that the latter instances are due to the presence of attenuated or weakened organisms or finely dispersed organisms (not in clumps as emulsions of cultures often are) which are more easily bathed in the acid and thus are retarded in growth or killed. It is possible also that the choosing of so called typical purulent flecks for the making of smears favors the finding of acid-fast bacilli, whereas the random method of taking up 1 c.c. of sputum for the culture procedure may by chance not include such material and hence result in a negative culture.

At one stage in this series the temperature of the incubator rose to about 40° C. A number of cultures became partially desiccated, the 15 c.c. of 5 per cent glycerin bouillon even disappearing. This presents an uncalculable source of error against the culture method. Despite careful impregnation of the culture tube cotton plugs one-half with vaseline-paraffin mixture, a number of cultures became dry, even at 37° C., and these contribute further to this error. "Airing" of the cultures was carried out until the danger of contamination became apparent and until it occurred to us that adding fresh air to a bare culture medium surface did not contribute anything to the growth of organisms possibly present. It was, therefore, discontinued.

Ageing of the sputum may be another unfavorable factor, probably negligible, since all specimens were immediately stored in the ice box awaiting culture, thus preventing putrefaction due to contaminants. One batch of 30 specimens (in February) was kept overnight at room temperature, yet

these grew out 4 cultures that were negative sputum smears, and only 2 contaminated specimens occurred in the lot

One specimen of sputum in the series from a single patient is of particular interest since it grew out a single colony on the crystal violet potato medium, while exhaustive examination of 10 different stained smears was fruitless in revealing acid fast organisms. In 8 instances less than 3 colonies appeared on the potato slant from heavy inoculation of the acid treated sputum. In only 3 of these specimens were acid fast bacilli seen in the smear. The scarcity of growth probably explains why the organisms were missed in the smear examination in the majority of these sputums. The advantage of the culture in these specimens is apparent.

The earliest gross appearance of colonies of tubercle bacilli from cultured sputums occurred in fifteen days, while the average length of time required for all cultures was thirty seven days. Examinations for new growth were made approximately every four or five days. Had it been possible to examine each culture every day it is probable that the average time of appearance would have been considerably reduced.

Cerebrospinal fluid obtained at autopsy from a case of tuberculous meningitis and untreated with sulphuric acid yielded an abundant growth on the potato medium in twenty four days. Similarly, tuberculous lung tissues from human autopsy material and from guinea pigs readily grew tubercle bacilli when subjected to the usual technique of culture. It is suggested that suspected tuberculous exudates, obtained by an aseptic technique, be cultured directly on the crystal violet potato medium as well as after acidification as a doubly sure method of detecting the organisms.

Stock cultures of tubercle bacilli may be transferred and subcultured in definitely on the crystal violet potato medium, provided the culture material be kept adequately moistened. If allowed to dry the organisms soon become nonviable. The crystal violet does not seem to impair cultures maintained in this manner.

Pathogenicity of Cultures—A point hitherto undetermined in reports in the literature on the application of the sulphuric acid crystal violet potato medium culture method is that relating to the pathogenicity of the acid fast bacilli obtained from the sputum or other sources, after isolation on the medium.

TABLE IV

DISPOSITION OF CULTURES OF TUBERCLE BACILLI OBTAINED FROM SPUTUM BY THE CORPER UYEI METHOD

Cultures dried, no growth on subculture, no animal inoculation	2
Cultures dried, old (2½ 6 months), no growth on subculture (in one) animal inoculations twice negative	3
Cultures viable animal inoculations successful in producing tuberculosis (acid fast bacilli recovered in 30)	33
Positive cultures (acid fast bacilli characteristic colonies etc)	38

Table IV is a summary of the results of guinea pig inoculation of samples of the cultures obtained in the series. In all instances in which the organisms remained viable after isolation from sputum the subsequent injection of

TABLE III

VARIATIONS IN AGREEMENT OF DIAGNOSES BY SMEAR AND BY CULTURE (ON THE BASIS OF UNCONTAMINATED CULTURES)

MONTH	SPECIMENS		AGREEMENTS				DISAGREEMENTS			
	TOTAL	UNCON- TAMIN- ATED ON CULTURE	NEG CULT NEG SMEAR	POS CULT POS SMEAR	TOTAL	PFR CENT	POS CULT NEG SMEAR	NEG CULT POS SMEAR	TOTAL	PER CENT
1928										
September	45	39	36	2	38	97.44	1	0	1	2.56
October	88	73	64	6	70	95.88	3	0	3	4.12
November	59	54	43	6	49	90.74	2	3	5	9.26
December	35	31	26	3	29	93.55	0	2	2	6.45
1929										
January	18	18	16	2	18	100.00	0	0	0	0.00
February	57	55	44	7	51	92.74	4	0	4	7.26
March	48	48	46	2	48	100.00	0	0	0	0.00
Totals	350	318	275	28	303	95.28	10	5	15	4.72

In Table III it may be noted that in several instances stained smears of the sputum revealed acid-fast bacilli whereas in the subsequent culture on the crystal violet-potato medium no growth of tubercle bacilli was obtained. The explanation for the failure of these specimens to grow colonies of the organisms is not clear. Malkani notes that a positive sputum may give growth on one occasion and at other times may not and admits his inability to offer an explanation. For the same reason, that he observes that ordinary cultures of tubercle bacilli when subjected to acid digestion frequently grow out, and previously positive sputums do not result in growth by culture, it is possible that the latter instances are due to the presence of attenuated or weakened organisms or finely dispersed organisms (not in clumps as emulsions of cultures often are) which are more easily bathed in the acid and thus are retarded in growth or killed. It is possible also that the choosing of so-called typical purulent flecks for the making of smears favors the finding of acid-fast bacilli, whereas the random method of taking up 1 c.c. of sputum for the culture procedure may by chance not include such material and hence result in a negative culture.

At one stage in this series the temperature of the incubator rose to about 40° C. A number of cultures became partially desiccated, the 15 c.c. of 5 per cent glycerin bouillon even disappearing. This presents an uncalculable source of error against the culture method. Despite careful impregnation of the culture tube cotton plugs one-half with vaseline-paraffin mixture, a number of cultures became dry, even at 37° C., and these contribute further to this error. "Airing" of the cultures was carried out until the danger of contamination became apparent and until it occurred to us that adding fresh air to a bare culture medium surface did not contribute anything to the growth of organisms possibly present. It was, therefore, discontinued.

Ageing of the sputum may be another unfavorable factor, probably negligible, since all specimens were immediately stored in the ice box awaiting culture, thus preventing putrefaction due to contaminants. One batch of 30 specimens (in February) was kept overnight at room temperature, yet

COMPARISON OF KOLMER WASSERMANN AND KAHN TESTS AND DARK FIELD EXAMINATION IN PRIMARY SYPHILIS*

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EXCEPT for isolated instances largely confined to the more recent reports, the literature on the Kahn test consists chiefly of statistical studies comparing its percentage of positivity with that of various types of complement fixation tests. The past year or so has finally brought forth papers comparing the reactions obtained in the Kahn and Wassermann tests with the actual status of the individual patient with regard to syphilitic infection. We have found only four studies of the reliability of the Kahn test in primary syphilis comprising a total of 240 cases.

Keim and Kahn¹ report 47 cases of primary syphilis of which 17 were negative with both the Kahn and Wassermann tests (eighteen hour ice box fixation), of the other 30 27 gave strongly positive Kahn tests and three were weakly positive. 26 gave strongly positive Wassermann tests, one a weakly positive and three were negative.

Houghton et al.² report 38 cases of which 4 were negative with both tests, 34 positive with the Kahn test and 26 with the Wassermann test (time of fixation not given).

Willett and Nagle³ in a study of the relative value of the Kahn test and dark field examination in 105 cases of early syphilis report a total diagnostic accuracy of the dark field examination in the first week of 76 per cent, the Kahn 56 per cent. In the second week the dark field examination showed 60 per cent positive, the Kahn test 90 per cent and in the third week the dark field examination 50 per cent, and the Kahn 90 per cent. In the fourth week the dark field examination gave 50 per cent, the Kahn test 88 per cent, while subsequent weeks showed the dark field examination 25 per cent and the Kahn test 100 per cent positive.

McIntyre and Gilman⁴ report 50 cases of primary syphilis with relative agreement between the Kahn and Kolmer tests in 83 per cent, absolute agreement in 68.7 per cent and no agreement in 17 per cent. Of the discrepancies 4.4 per cent had positive Kahn tests and negative Kolmer tests and 12.6 per cent positive Kolmer tests and negative Kahn tests.

We are reporting herewith a series of 1061 cases of proved early syphilis. All of the patients had dark field examinations of the initial lesion, Kolmer blood Wassermann, and Kahn tests. In none of these cases does the diagnosis rest solely on clinical evidence. We saw 44 per cent of these patients in the

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first week of then infection, 28.4 per cent in the second, 12.2 per cent in the third, 4.2 per cent in the fourth, and 1.2 per cent in the fifth or subsequent weeks

Of the total series, regardless of the duration of the lesion, 47.3 per cent showed positive dark-field examinations and negative serologic examinations, 35.1 per cent were positive both by dark-field and serologic examinations (Kolmer test, Kahn test, or both) while 14.6 per cent had negative dark-field examinations but positive serologic examinations. At the time of the first examination 3 per cent gave negative results with all methods, the diagnosis being made by subsequent tests. In other words a total of 82.4 per cent of these cases had positive dark-field examinations and only 49.7 per cent had positive blood tests.

The relative value of the dark-field examination becomes still more striking when these figures are analyzed by weeks. Of those patients seen in the first week after the appearance of the initial lesion, 7.1 per cent had positive dark-field examinations only, 21.5 per cent had both positive dark-field examinations and serologic tests, while 5 per cent showed positive serologic examinations only, a total accuracy of 92.5 per cent for the dark-field examination and 26.5 per cent for the blood tests. In the second week we found 45.5 per cent positive, only with the dark-field examination, 41.3 per cent with both dark-field and serologic examinations positive, and 9.3 per cent with positive serologic and negative dark-field examinations, a total of 87.3 per cent positive dark-field and 51.1 per cent positive serologic examinations. The third week gave 25.8 per cent positive with the dark-field examination only, 49.2 per cent positive with both tests and 21.9 per cent positive only with the blood tests, a total accuracy of 75 per cent for the dark-field examination and 71.1 per cent for the blood tests. By the fourth week we found only 4.4 per cent positive with the dark-field examination alone, 47.8 per cent positive with both tests, and 41.3 per cent with serologic examination only, the accuracy of the dark-field examination being 52.2 per cent and the blood tests 89.1 per cent. In the fifth and subsequent weeks there were no patients with positive dark-field examinations and negative blood tests, 51.2 per cent having both tests positive and 47.9 per cent positive solely with the blood tests. The "misses" remain about 3 per cent throughout the entire first four weeks. It is therefore not until the third week that the serologic examination begins to approach the dark-field examination in diagnostic accuracy.

This series also represents an opportunity to study the relative sensitivity of the Kahn and Kolmer tests in a fairly large, controlled group of known cases of early syphilis. Tabulating these again by weeks we find, in the first week, 1.9 per cent positive with both the Kahn and Kolmer tests, 5 per cent positive only with the Kahn test, and 2.5 per cent only with the Kolmer test. In the second week there were 44.8 per cent positive with both tests, 3.3 per cent only with the Kahn test, and 3.0 per cent with the Kolmer test. In the third week 6.4 per cent were positive with both tests, 3.2 per cent with the Kahn test, and 3.9 per cent with the Kolmer test. By the fourth week 80.3 per cent gave positives with both tests, 8.8 per cent with the Kahn test only, and none with the Kolmer test (the relatively small number of cases in the fourth

TABLE I

SHOWING TOTAL NUMBER AND PERCENTAGE OF POSITIVE CASES WITH DIFFERENT METHODS AT VARIOUS PERIODS AFTER THE APPEARANCE OF THE PRIMARY LESION

DAYS SINCE APPEARANCE OF THE PRIMARY LESION	17 DAYS		8-14 DAYS		10-11 DAYS		22-28 DAYS		28-30 DAYS		TOTAL	
	NO	PER CENT	NO	PER CENT	NO	PER CENT	NO	PER CENT	NO	PER CENT	NO	PER CENT
Positive dark field examination	330	71	137	45.5	33	25.8	2	4.4	0	0	502	47.3
Negative serologic examination												
Positive dark field examination	70	15	107	35.0	57	44.0	20	43.4	61	51.2	310	29.7
Positive Kolmer test												
Positive Kahn test												
Positive dark field examination	18	4	10	3.3	2	1.6	2	4.4	0	0	32	3.0
Positive Kahn test												
Negative Kolmer test												
Positive dark field examination	12	2.3	9	3.1	4	3.1	0	0	1	0.9	26	2.4
Positive Kolmer test												
Negative Kahn test												
Negative dark field examination	17	4	28	9.3	20	19.5	17	36.9	57	47.9	144	13.6
Positive Kahn test												
Positive Kolmer test												
Negative dark field examination	4	1.0	0	0	2	1.6	2	4.4	0	0	8	0.8
Positive Kahn test												
Negative Kolmer test												
Negative dark field examination	1	0.2	0	0	1	0.8	0	0	0	0	2	0.2
Negative Kahn test												
Positive Kolmer test	15	3.5	10	3.3	4	3.1	3	6.5	0	0	32	3.0
Negative dark field examination												
Negative serologic examination												
Number and per cent of cases seen at different periods	467	44	301	28.4	123	12.2	46	4.2	119	11.2		1061

week group gives these figures a fictitious accuracy) In the fifth week there was one patient who gave a positive only with the Kolmer test, the other 118 being positive with both tests Altogether there were 33.3 per cent positives with both Kahn and Kolmer tests, 3.8 per cent positive with the Kahn test only, and 2.6 per cent positive with the Kolmer test only So far there has seemed to be but little if any difference in sensitivity of the two tests provided all reactions of any degree are counted as positives The question of the diagnostic significance of weak positives will be discussed later in this paper

In this series there were 113 patients showing a marked discrepancy between the results obtained with the two tests Of these 47 gave a reaction with both the complement-fixation and precipitation methods differing markedly in degree, 31 having strongly positive Kolmer tests and weak Kahn tests and 16 strong Kahn tests and weak Kolmer tests Of the 27 cases with positive Kolmer tests and negative Kahn tests, 11 were +++ or ++++ positive while of the 39 patients with positive Kahn tests and negative Kolmer tests, there were 14 with ++++ or +++ positive reactions, 25 being + or ++ in strength The actual dependence to be put on such + or ++ positive Kahn tests becomes somewhat obscure when we find that in an additional 524 patients with venereal sores who were proved to be nonsyphilitic, there were 15 who showed at some time in the serologic investigation of the etiology of their infection Kahn reactions of varying strengths but who did not develop a positive Kolmer Wassermann test within a period of more than thirty-five days after the onset of the lesion nor subsequent clinical evidence of syphilis They were not treated for syphilis during this time If the Kahn test alone had been taken as the diagnostic criterion, we would have had an incidence of false positive diagnosis in the negative cases of 3 per cent The incidence of weak positive Kahn tests and negative Kolmer tests in the positive series is 2.5 per cent Whether these aberrant positives represent true false positive reactions or technical errors, we are not attempting to say These tests were run by experienced technicians, checked by one of us and all discordant results repeated The cases reported here represent only those obtained on repeat tests In the same group of cases we obtained one Kolmer test reading of 1-0-0-0-0 which proved not to be a case of primary syphilis

CONCLUSIONS

- 1 The most reliable single diagnostic procedure in early syphilis is the dark-field examination
- 2 Serologic examinations approach the dark-field examination in accuracy only after the third week of the primary lesion
- 3 The multiple approach (dark field and serologic examinations) gives the most information and the highest diagnostic accuracy (97 per cent)
- 4 In this series there is no appreciable increase in specificity nor earlier positivity of the Kahn test over the Kolmer-Wassermann method
- 5 To obtain the highest technical accuracy both Kahn and Kolmer tests should always be made, checking both where a discrepancy occurs

6 The Kahn test is more subject to technical error and weak positive results should not be taken as evidence that a venereal lesion is necessarily syphilitic

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A TIME-SAVING DEVICE FOR WASHING TEST TUBES*

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WHENEVER large quantities of test tubes are used for laboratory procedures, for instance in the performance of the Wassermann test, their emptying and rinsing, preliminary to the thorough cleansing, is very time-consuming. The usual procedure is to take out with one hand about two or three test tubes at a time from the rack, to place them in the other hand until a handful of about six or eight have been collected, then to empty them by turning upside down, to fill them with water again, and to repeat that procedure as many times as necessary. To empty and rinse the test tubes of a rack holding about seventy-two tubes, the above procedure has to be repeated about ten times. Besides the loss of time, the handling of individual test tubes increases the breakage considerably.

To obviate the above difficulties, some laboratories use a rectangular piece of wire net, which is put on top of the filled test tube rack, it is held down on the sides with both hands and so the entire rack can be turned upside down.

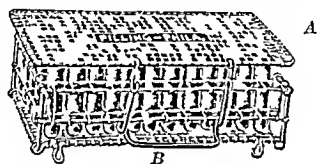


Fig 1

for the emptying and refilling of the test tubes. We have observed the use of such a wire net and tried it ourselves repeatedly. We noticed that the net has a tendency to slip and that a considerable amount of skill is necessary to handle it satisfactorily. If the procedure is not carried out properly, no time is saved, and the slipping of the net chips the edges of the test tubes. It occurred to us that the instability of the wire net could be removed by attaching it with two clamps to the shelf of the test tube rack.

As shown in the illustration, the apparatus consists of a rectangular net made of galvanized wire netting (A) with a broad wire clamp (B) on each side, catching at the middle shelf of the test tube rack. Any test tube rack provided with a middle shelf is satisfactory.

The apparatus permits turning the test tube rack with its contents upside down and emptying the tubes at once. The clamps hold the lid firmly and reliably. The apparatus is very easy to handle, it is simple in construction, and is readily made. The edges of the test tube mouths are not chipped, and the breakage is considerably decreased.

It is our experience that by the use of the above simple device valuable time is saved in a busy laboratory.

The George P. Pilling and Sons Company, Philadelphia, has cooperated in carrying out our designs.

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ATTEMPTS TO SECURE OBJECTIVE METHODS OF STUDYING MILD ANAPHYLAXIS*

By R D TEMPLETON AND W F BOLLENS CHICAGO ILL

PHYSIOLOGIC means of recognizing a mild anaphylactic shock are not well developed

Taking the guinea pig as the most susceptible animal Harvey and Templeton (1926) sought to determine the onset of anaphylaxis by observing the gross manifestations Egg white was used as the foreign protein and the time varied for the second injection from fifteen to twenty four days after sensitization If the shock was sufficiently severe, the gross symptoms were distinct, but in many instances the symptoms shown were not sufficiently different from the normal reactions of the control pigs to make this of diagnostic value A few attempts were made to study the contractions of the descending colon, but with very little success

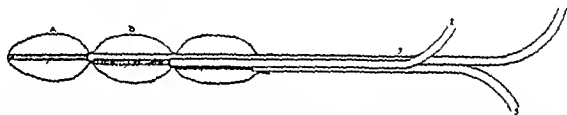


Fig 1—Triple balloon K ligations separating the balloon into three distinct balloons A B C, balloons formed by the ligations at A each balloon is independent of the other X small wire coil springs extending from the balloon through the rubber tube to 1 2 3 rubber tubes leading from balloons to manometers

At this time (January, 1927) we took up the work Normal contractions of the descending colon of several guinea pigs were recorded over a period of several weeks

In order to study the character of the colon contractions a system of three balloons was devised These tubes were so arranged that a single balloon could be drawn over them and tied off at three places to form the three independent balloons In order to prevent collapsing of the tubes and balloons and to give rigidity to the system, small wire coil springs were inserted into the tubes as shown in Fig 1 The tubes were fastened together in the form of a triangle with rubber cement and mastic The springs protrude about one inch from each rubber tube preventing collapse of the first balloon and keeping the other two balloons from sticking to the rubber tubing They also enable one to tie the balloons without constricting the rubber tubing

This method of study shows the character of contractions in the descending colon of guinea pigs to be peristaltic, mass, and probably pendular or segmentation, i.e., any one balloon initiating a contraction wave in either direction

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The normal activity shows considerable variation during a single test and also from day to day. In anaphylactic shock recognizable by the gross manifestations, the contractions of the descending colon are augmented but not sufficiently so to make this a satisfactory method for recognizing anaphylaxis.

A small balloon inserted in the vagina of a virgin pig under urethane anesthesia shows marked variations in the motor activity. Whether these variations are dependent upon the position of the balloon, the manometer pressure, the depth of anesthesia, or the estrus cycle, has not been determined. Considerable augmentation of the contractions is caused by histamine, but the normal variations are too great for using this as a method of recognizing mild anaphylactic shock.

The established uterine strip method and our partial success with the vagina led us to attempt methods of recording uterine activity *in vivo* without anesthesia. A Thury-Vella fistula (dog) was prepared, and one horn of the uterus was wrapped around the intestinal segment. After healing a balloon was inserted into the fistula so as to lie directly under the uterine loop, but the intestinal contractions are so great normally that the recognition on the tracing of uterine movements is practically impossible. However, when oxytocin* is injected contractions of the intestinal loop are almost completely stopped for a few minutes, and the tone is raised, Fig 2-A. This rise in tone is probably due to uterine contractions since it does not appear in fistulas not in contact with a uterine loop (Fig 2-B). The paralysis of intestinal movements by oxytocin is very temporary, and when the contractions return, further study of uterine activity is difficult.

Twenty days after sensitization the injection of 15 cc of fresh egg white into a dog with a utero-Thury-Vella fistula caused a slow rise in tone, beginning ten minutes after the injection, Fig 2-C. About forty minutes after this anaphylactic injection oxytocin was injected. The intestinal contractions were stopped, but the change in tone was very slight. The rise in tone following the injection of egg white was probably due to anaphylaxis. The failure of oxytocin to produce the typical tone change was probably due to the anaphylactic contraction of the uterus. While oxytocin diminished the tone of the intestine as it does normally, there was no sharp increase in tone due to uterine contraction. This is probably evidence that the uterus was already contracted to a maximum by the protein.

The work of Weitz and Volleis¹ on pregnant women, in which they were able to record rhythmic activity of the uterus, suggests that records of uterine action might be made from the uterus of dogs if lifted and sewed to the ventral peritoneum. An operation was performed in which the horns of the uterus were sewed to the peritoneum on each side of the midline. In one case an abdominal muscle was removed so as to produce a hernia in which the uterus could lie. Attempts were made to record uterine movements from this preparation by means of a frog lever attached to a straw which rested over the position of the uterus. Intestinal movements interfered to the extent that it was not possible to differentiate them from those due to uterine action.

In March, 1928, an operation was attempted, the idea of which had sug-

*The oxytocin used in this experiment was furnished by the Parke Davis Laboratories

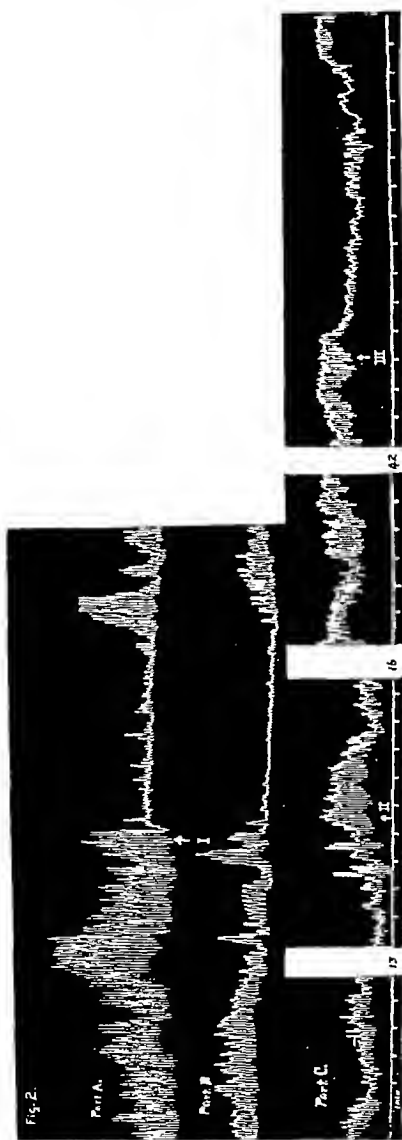


Fig. 2. —A, B simultaneous balloon tracing from a double Thiry Vella fistula (dog). I injection of 0.5 c.c. of oxytocin. A the uterine horns are wrapped around the intestinal loop. B intestinal loop used for control. C balloon tracing from Thiry Vella fistula around which the uterine horns are wrapped. II injection of 1 c.c. egg white (unabsorbed in the control). III injection of 0.5 c.c. of oxytocin.



Fig 3—4, *B* simultaneous tracing from the vagina and uterine exposure. *I* injection of 0.5 cc oxytocin. *A* balloon tracing from vagina within two inches of uterine bifurcation. *B* plethysmograph tracing from uterine exposure.

gested itself early in the work but which had been postponed in order to try what seemed to be simpler surgery, the idea being to bring the uterus entirely outside the body for study.

A loop of the uterus about 15 inches long, the point of bifurcation and about one half inch of the vagina were brought entirely outside the body. The blood vessels running parallel with the uterus were also brought out. The peritoneum, muscle and skin were closed underneath, care being taken not to compress the blood vessels. The surgery is very simple and the animal recovers promptly. Such a preparation is serviceable for experiments for several months and probably indefinitely.

It was possible to place a small plethysmograph around the exposed uterine loop and record the spontaneous movements of the intact organ. A normal record from this uterine preparation showed pulse, respiration and spontaneous uterine movements. In our preparation, contractions caused a buckling of the exposed loop raising the water level in the manometer. A balloon inserted through the vulva, to within two inches of the uterine bifurcation showed spontaneous activity, but not apparently correlated with the uterine movements.

The injection (intravenous) of oxytocin (Fig 3) caused partial tetany of the uterus within ten seconds, while a contraction of the vagina two inches from the uterine bifurcation was not elicited until one minute after the injection. The effect of oxytocin on the uterus can be clearly seen for at least one hour. The pulse which is obliterated by the partial tetany begins to return in about fifteen minutes. The duration of the vaginal contractions is only about eight minutes.

A subcutaneous injection of 0.06 mg of ergotamine caused a partial tetany of the uterus within five minutes. This tetanic condition lasted about eight minutes, followed by augmented contractions for several hours. At the onset of tetany the pulse disappeared and did not return for an hour or more. Oxytocin did not give a characteristic effect until forty eight hours had elapsed following the ergotamine injection when normal activity was restored.

SUMMARY

1 Contractions of the descending colon in guinea pigs studied by the triple balloon method, are augmented during anaphylaxis only when the shock is pronounced.

2 Normal variations in the activity of the descending colon and vagina of guinea pigs are opposed to their use as indicators of anaphylaxis.

3 Graphic records of uterine activity can be obtained from the exposed uterus of dogs and the animal kept in good condition several months (probably indefinitely).

4 The exposed uterus of the unanesthetized animal offers good opportunity for study of the uterus in situ.

We are indebted to Dr. A. J. Carlson under whose directions this work was conducted and wish to thank Dr. C. S. Smith and Dr. O. H. Horrall whose surgical assistance was invaluable.

REFERENCE

THE SEDIMENTATION VELOCITY OF ERYTHROCYTES IN THYROTOXICOSIS*

By J M MORA, M D,† AND J T GALIT, M D,‡ CHICAGO, ILL

IT IS somewhat more than a decade since Robin Fahraeus¹ first reported his observations on the sedimentation velocity of red blood cells. The widespread interest aroused by this apparently simple yet extremely complex phenomenon was evidenced by the remarkably voluminous literature that accumulated in this brief period. The original observations dealt with the study of the reaction in pregnancy, but it has since been carefully studied in a great variety of conditions.

The sedimentation speed of the red corpuscles in man is subject to physiologic variations according to age, sex, and, in women, to the absence or presence of pregnancy. (The latter is the only physiologic condition in which the sinking velocity is increased.) The lowest figures are obtained in the newborn, in adults, men generally show considerably lower figures than women. During gestation, the increased sedimentation velocity becomes apparent in the second month, reaches its highest figures at term then rapidly recedes during the puerperium. It is more or less increased in all acute general infections and appears to be of considerable value as a source of information as to the occurrence of complications. In chronic infectious diseases, such as syphilis and tuberculosis, the sedimentation speed is quite markedly increased. The field of internal medicine in which this reaction has been most fully investigated and the most important results obtained is pulmonary tuberculosis.

As first noted by Westergren² and since corroborated by other investigators, the sedimentation speed may reflect better than the body temperature the intensity of the tuberculous process. Gynecologists have found the reaction of value in differentiating pelvic inflammatory lesions from neoplastic and other lesions. To quote Fahraeus³ "It may be said that a reduction in the suspension stability of the blood is one of the most common general reactions of the organism in disease, perhaps the most common. In this respect it may best be compared with such reactions as pyrexia and leucocytosis. On account of its completely nonspecific character, the reaction has, as a rule, proved less instructive in respect to the diagnosis of the disease, than as regards the activity or intensity of the morbid process."

The hydrodynamics of the sedimentation reaction are very complex. According to Fahraeus³ the factors which influence the sinking velocity are the concentration of the suspension (i.e., the relative number of corpuscles), the radius of the particle, and the tendency of the red blood corpuscles to form

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aggregations or rouleaux. The latter is said to determine the sedimentation speed. Increased rouleaux formation is caused mainly by increase in the serum globulin and fibrinogen. The lipoids of the plasma have also been considered of importance, as have the gases of the blood. Experiments *in vitro* by Leendertz⁷ have shown that the sinking velocity is increased if oxygen is bubbled through citrated blood, whereas carbon dioxide decreases it.

The most commonly used methods of determination are those of Fabreus⁸ and Westergren (the "distance method") in which the sedimentation speed is obtained by measuring the distance passed by the uppermost layer of red cells from the upper meniscus of the fluid column in a given time and that of Lanzemeier⁹ (the "time method") in which the time in minutes is taken that is required for the corpuscles to sink a certain distance marked on a specially calibrated tube. Using the latter method the normal time has been found to average 850 to 1000 minutes for women and 1000 to 1200 minutes for men (Lanzemeier⁹, Friedlander¹⁰, Loh⁶). Briefly the technique consists of mixing 0.2 c.c. of freshly prepared 5 per cent sodium citrate solution, with 0.8 c.c. of blood and noting the time required for the cells to sink 18 mm.

The influence of thyrotoxicosis on the sedimentation velocity seems to have been studied but little. Ueno¹¹ in 1926 studied 19 cases. In 8 the rate was markedly increased, in 6 slightly increased, in 3 the rate was normal and in 2 cases it was decreased. DeCourcy¹² reported 7 cases of mild hyperthyroidism in which the sedimentation rate was increased. Tschernosvonskaya¹³ recently studied 42 cases, and without exception the rate was increased. He believes that the sedimentation velocity parallels the severity of the disease process.

We studied 30 cases of thyrotoxicosis before and after operation. (A control series of 20 other surgical cases was also included, these consisting of 11 inguinal herniotomies, 3 ventral herniotomies, 2 hysterectomies and 4 thyroidectomies for nontoxic adenomatous goiter.) In 11 of the 30 cases we were able to note the effect of the administration of iodine. All of the 30 cases showed a definite increase in the sedimentation velocity before operation but the operative relief of the hyperthyroidism did not uniformly affect the sedimentation speed. In 6 of the 11 cases (in whom the rates were taken before and after the administration of iodine) there was a decreased sedimentation speed after the period of iodization coincident with a drop in the basal metabolic rate. The other 5 cases showed an increased rate during the same period. After maximal thyroidectomy there was a decrease in the sedimentation speed in 14 of the 30 cases, in 13 of the cases there was an increased rate and in the 3 remaining cases the rate remained about the same as before operation. It was rather confusing to note the increased rate after operation in nearly one half of the cases, particularly since 8 of these patients were definitely hypothyroid. We are at a loss to explain this phenomenon. There appeared to be little parallelism between the sedimentation speed and the basal metabolic rate. It will be observed that all the postoperative velocities were rather rapid, and as these cases were studied from one to six months following thyroidectomy, it may be that these patients require a much longer time to

regain their normal state than we ordinarily suppose. On the basis of this study it appears that the sedimentation rate is of little clinical value in thyrotoxicosis.

SUMMARY

The sedimentation speed of erythrocytes was studied in 30 cases of thyrotoxicosis, by the Linzenmeier method, before and after thyroidectomy. The rate was increased in all cases before operation. The administration of iodine decreased the speed of sedimentation in 6 out of 11 cases, and increased it in 5 cases. Thyroidectomy was followed by an increased rate in 13 cases, and by a decreased speed in 14 cases. There seemed to be little parallelism between the sedimentation velocity, the basal metabolic rate, and the clinical picture.

We desire to acknowledge our indebtedness to Mrs. V. Haste and Mrs. B. Rieger, for their aid in this work.

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A TECHNIC FOR OPERATIONS ON SEVERAL SMALL ANIMALS AT ONE TIME BY USE OF AMYTAL ANESTHESIA*

By ROBERT L. JOHNSTON, M.D., CLEVELAND, OHIO

THE technic herein described enables one to carry on without the assistance of an anesthetist three to five aseptic surgical operations on small animals such as rats, guinea pigs or rabbits, with a single sterile preparation. By this technic as many as ten animals can be operated upon within a single morning.

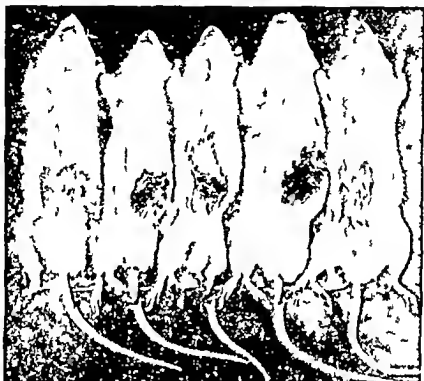


Fig. 1—Simultaneous operation on five rats under amytal anesthesia.

Stage 1. Local application of iodine at site of injection of amytal. Note attachment of animals to operating board by means of adhesive over legs.

ANESTHETIC

Amytal (Lilly) is purchased in 50 gm quantities and prepared according to the directions of the manufacturer, namely, to one gram of amytal 8.85 cc of N/2 sodium hydroxide solution is added with stirring the mixture is placed over a boiling water bath and stirred frequently. Solution occurs within from ten to twenty minutes with the formation of amytal sodium, or sodium iso amyl ethyl barbiturate. When dissolved and made up to a 10 per cent solution it is ready for use on large animals. For use on small animals, however, it should be further diluted to a 1 per cent solution otherwise the danger of overdosage is increased tenfold. Only fresh solutions should be used, clouded solutions should be discarded.

From the Cleveland Clinic, Cleveland, Ohio.
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TECHNIC

The animals are weighed and the appropriate amount of the 1 per cent solution calculated for each animal. With a fresh stock of amytal, 0.060 gm per kilogram is adequate to maintain complete surgical anesthesia for two or more hours. Rabbits often require 0.075 gm per kilogram, sometimes more.

An assistant holds the animal while a 2 per cent solution of iodine is applied to the abdomen at the site chosen for injection. The computed dose is given intraperitoneally from a Luer syringe, using a small hypodermic needle. Usually by the time the last of five animals is injected (four or five minutes) the first animal is ready to be attached to the operating board which consists of a piece of wood board about 7 by 10 inches in size. The animal's legs can be quickly attached by means of small strips of adhesive tape, overlapping in the manner shown in Fig. 1. By the time all the animals are attached to the board surgical anesthesia should be complete in all.



Fig. 2—Simultaneous operation on five rats under amytal anesthesia.
Stage 2 (Right) Application of barium sulphide to abdomen.
Stage 3 (Left) Warm wet cloth applied over barium sulphide.

The field of operation is next sprinkled liberally with barium sulphide and then covered with warm wet cloths to effect saturation with water, as shown in Fig. 2. Within five minutes, the barium sulphide can be swabbed off with wet gauze, bringing with it all the underlying hair (Fig. 3). The skin is then dried with gauze and is painted with the usual alcoholic solution of iodine. The operator scrubs, puts on a sterile gown and drapes all the animals at one time, after placing pieces of gauze between them (Fig. 4). The desired operative procedures are now carried out (Fig. 5) and the closures made, peritoneum with muscles then the skin, by means of silk sutures. We have found the continuous, hidden, subcuticular skin suture very satisfactory for work on rats and rabbits. Occasionally, however, we use interrupted sutures. Usually the skin sutures are extruded, but those in the muscular layer are rarely extruded, as shown by subsequent autopsy.

Occasionally, an overdose is given, as a result of struggling by the animal

while the injection of the anesthetic is being made. Also, sometimes when operative procedures are prolonged, one or more of the rats will begin to recover, so that a second injection is required with resultant overdosage. For these reasons when animals are operated upon in such numbers, there is a mortality of from 10 to 20 per cent. This mortality, however, is negligible when we take into consideration the time saved by the use of a method which



Fig 3—Simultaneous operation on five rats under amytal anesthesia
Stage 4 Field of operation after removal of barium sulphide.

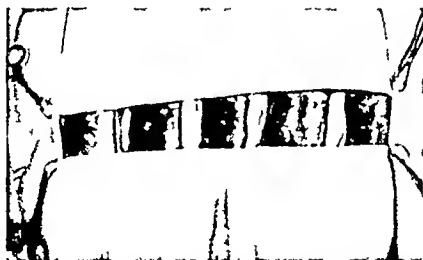


Fig 4—Simultaneous operation on five rats under amytal anesthesia
Stage 5 Iodine applied to field of operation and drapes applied

makes it possible for one operator to perform as many as twenty operations in a single day without an anesthetist.

Although this paper is not intended to be a study of amytal anesthesia but rather a description of its uses in connection with experiments on small animals, nevertheless it may be well to draw attention to the works of the following authors as examples of the direct study of this substance as an anesthetic.

Page and Coryllos¹ described a method of preparation of amytal solutions

using ethylene glycol as the solvent and preservative. In their experience, a dosage of 45 to 50 mg per kilogram is most suitable for intravenous administration in dogs. They employ a dosage 15 to 20 mg higher for intraperitoneal administration as advocated by Edwards and Page.²

Swanson and Page³ state that the intravenous minimal effective dose for amytal in cats is 45 mg per kilogram as compared with 155 mg per kilogram for barbital. They find the induction speed an hour shorter with amytal than with barbital. They give statistics of their own work, together with statistics given by other authors, which show the minimal effective dose as compared with the minimal fatal dose in over 500 animals of various species.

In a series of ten experiments on rabbits, Underhill and Spurr⁴ found that eight gave evidence of distinct hyperglycemia following doses ranging from 50 to 100 mg per kilogram, whether the amytal was given by mouth,

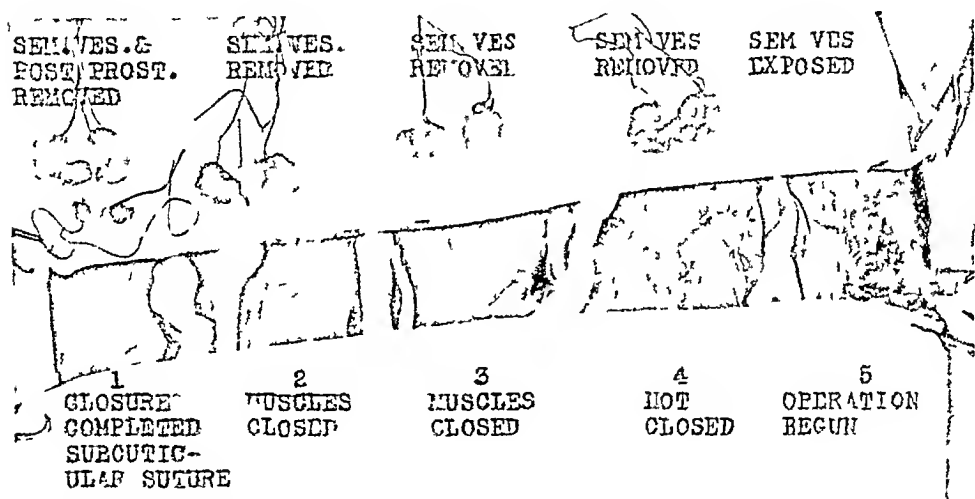


Fig. 5—Simultaneous operation on five rats under amytal anesthesia. Illustration of method of closure.

subcutaneously, or intraperitoneally. They call attention to the fact that Weiss⁵ has shown that amytal produces hyperglycemia in the cat and the dog.

Hines, Boyd, and Leese⁶ found that dogs under amytal anesthesia have a decreased capacity to assimilate injected glucose.

Hines, Leese, and Barei⁷ compared the glycogen content of liver and muscle in normal animals with that in animals anesthetized with amytal, using eight dogs. Three hours following the injection of glucose solution, they found the glycogen content of muscle to be the same as prior to amytal anesthesia. They found, however, that the liver glycogen was increased to double the amount in the unanesthetized animals.

Investigators using amytal anesthesia on dogs are familiar with a characteristic shivering which occurs when the anesthesia begins to diminish. I have noticed this phenomenon commonly in dogs, but never in rats or rabbits. It has been noted that these smaller animals, which do not shiver under amytal anesthesia, very readily lose their body heat so that in prolonged operative

proceedures the superficial tissues actually feel definitely chilly to the touch. It is believed that postoperative complications are reduced materially by, actually combating, this heat loss. The mechanism of this heat loss is apparently as follows: carbohydrate metabolism is suppressed, as evidenced by the existence of hyperglycemia. Carbohydrate formation or glycogenesis is not suppressed as much as the glucose utilization as evidenced by the increased glycogen content of the livers of animals anesthetized with amytal as compared with the livers of unanesthetized animals. It follows that suppression of glucose utilization lowers the heat production which is combustion of both carbohydrates and fats. In dogs, shivering combats this heat loss. In animals which do not shiver and which are smaller, the heat loss is greater due to the absence of shivering, and also to the larger relative body surface for heat radiation. Uncombated heat loss subjects the animals to postoperative complications especially to pulmonary complications.

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NOTE ON THE DETERMINATION OF INORGANIC PHOSPHATE OF SERUM AND SPINAL FLUID ON THE SUPERNATANT FLUID FROM CALCIUM ESTIMATION*

By CLARE LOWENBERG, B S , AND MARJORIE R MATTICE, A B , New York, N Y

IT IS often difficult to obtain sufficient blood serum or spinal fluid for determination of both calcium and inorganic phosphate in very young children. Gunther and Greenberg¹ recently published a modification of the Fiske and Subbaw² inorganic phosphate determination applicable to the supernatant fluid from the Clark-Collip modification of the Kramer-Tisdall³ calcium determination. Following their suggestions we have determined inorganic phosphate by the Benedict and Theris⁴ method on the supernatant fluid from the calcium determination.

PROCEDURE

In a 15 c c conical centrifuge tube place 2 c c serum, 2 c c distilled water, and 1 c c of 4 per cent ammonium oxalate. Mix thoroughly by gentle shaking. After thirty minutes, centrifuge. Decant the supernatant fluid into a wide mouth tube and invert the centrifuge tube to drain, proceeding as usual with the calcium determination.

To 4 c c of the supernatant fluid add 2 c c of water and 4 c c of 20 per cent trichloroacetic acid. Mix thoroughly and filter after ten to twenty minutes. Measure 5 c c of the clear filtrate into a Myers' sugar tube, add 1 c c water, 2 c c 0.1 N potassium permanganate, and without delay 1 c c molybdic sulphuric acid reagent and 1 c c bisulphite hydroquinone reagent. Prepare the standard as usual. Mix, stopper loosely, and heat in a boiling water-bath for ten minutes. Cool and compare in the colorimeter, setting the standard at 15.

Calculation —

$$\frac{15}{R} \times 0.025 \times \frac{100}{0.8} = \text{mg P} / 100 \text{ c c serum}$$

The 4 c c supernatant fluid used represent 16 c c serum. Five c c of the trichloroacetic acid filtrate therefore represent 0.8 c c serum. The strength of standard used is 0.025 mg phosphorus in 3 c c.

The procedure for estimation of inorganic phosphate in spinal fluid on the supernatant fluid from the calcium is similar to that in serum, except for a change in proportions made necessary by the smaller amount of inorganic phosphate present in spinal fluid.

Add 1 c c of ammonium oxalate and 1 c c of water to 2 c c of spinal

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fluid Mix 3 c.c. of the supernatant fluid and 2 c.c. of 20 per cent trichloroacetic acid. After ten minutes filter until clear through a small filter paper (not over 5.5 cm). Take 4 c.c. of the filtrate, 3 c.c. of 0.1 N permanganate, and 1 c.c. each of water, molybdic acid and hydroquinone reagents for the color development.

Calculation —

$$\frac{15}{R} \times 0.025 \times \frac{100}{1.2} = \text{mg P / 100 c.c. spinal fluid}$$

DISCUSSION

In Table I are shown the results obtained by determination of inorganic phosphate on serum and on supernatant fluid from calcium determination. The variation is negligible in practically every case.

TABLE I

SPECIMEN	INORGANIC PHOSPHORUS OF SERUM MG / 100 C.C.	INORGANIC PHOSPHORUS OF CALCIUM SUPERNATANT FLUID MG / 100 C.C.	DEVIATION FROM SERUM VALUE MG / 100 C.C.
1	4.1	4.0	-0.1
2	3.0	4.0	+0.1
3	3.9	3.9	0
4	4.6	4.8	+0.2
5	3.3	3.3	0
6	3.4	3.4	0
7	4.0	4.0	0
8	6.0	5.8	-0.2
9	2.1	2.2	+0.1
10	3.9	3.9	0
11	4.8	4.6	-0.2
12	3.6	3.7	+0.1
13	5.9	5.8	-0.1
14	3.1	3.3	+0.2
15	3.4	3.4	+0.1
16A	6.7	6.3	-0.4
16B	7.0	7.2	+0.2

In Table II are shown the results obtained by determination of inorganic phosphate of spinal fluid and in supernatant fluid from calcium determination.

TABLE II

SPECIMEN	INORGANIC PHOSPHORUS OF SPINAL FLUID MG / 100 C.C.	INORGANIC PHOSPHORUS OF CALCIUM SUPERNATANT FLUID MG / 100 C.C.
1	14	15
2	15	17
3	13	13
4	15	14
5	16	17
6	16	16
7	16	16
8	18	18
9	16	16

Determination of inorganic phosphate on the supernatant fluid from the calcium determination without the addition of permanganate (Table III) gave low results, the error increasing with larger amounts of phosphate.

TABLE III

SPECIMEN	INORGANIC PHOSPHORUS	INORGANIC PHOSPHORUS OF	DEVIATION FROM
	OF SERUM	CALCIUM SUPERNATANT FLUID	SERUM VALUE
	MG /100 C C	MG /100 C C	MG /100 C C
L	2.9	2.5	-0.4
M	5.2	3.6	-1.6
J	8.0	4.9	-3.1

Briggs⁵ states that oxalates and citrates in such amounts as are used to prevent clotting of blood do not interfere with the acid development of the blue color, and thus plasma may be used instead of serum for the determination of inorganic phosphate. The two cc of 0.1 N permanganate added is nearly the theoretical amount required to oxidize the oxalate present.

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GASTROENTEROLOGIC TABLE WITH A SPECIAL DRAINAGE OUTFIT*

LY MOSES EINHORN, M D, NEW YORK

WHILE engaged in the practice of gastroenterology, I have been in a position to observe closely the various types of tables used in this work. My observations point to the fact that there is at present no standard table physicians using whatever is nearest at hand, such as a surgical table a house couch, or any other common type. Each of these has its disadvantage, some being too high or too low for the convenience of the examiner others too soft or too hard for the patient's comfort. None of the tables mentioned allows for an adjustable headrest.

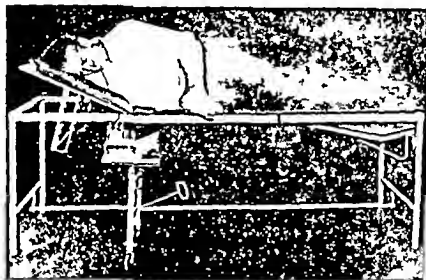


Fig 1

In using the tables for drainage work a separate chair placed on a lower level than the table is necessary for the arrangement of the test tubes. Any movement on the part of the patient may thus spill the contents of the tubes and disturb the work of the drainage apparatus. In doing rectal work an additional side table is necessary for the instruments.

Noting the disadvantages, and realizing the need of a standard table I constructed a special table (Fig 1) in order to eliminate these difficulties. The table is six feet long, two feet wide and two feet, two inches high and is constructed with a metal top supported by four legs. It is covered with a soft comfortable leather pad and has an adjustable head support. The table is reinforced in the middle by a longitudinal bar, to which is attached a special drainage outfit. The upper section of this outfit is composed of three parts, a special test tube rack with perforations to hold the tubes securely in place, a small box to contain the drainage supplies and a concave support for the syringe. The whole is supported by an iron bar, which is telescoped in a

tube, at the base of which is a large wheel. Extending from this tube, and perpendicular to it, are two parallel rods, also telescoped in a U-shaped iron tube, with a small wheel at its base. This U-shaped tube connects the entire drainage outfit to the longitudinal bar which reinforces the table. Also, there is a handle convenient for sliding it into the required position. Several small screws are attached to the outfit to allow for the adjustment thereof.

For rectal work, there is a special tray to hold the necessary instruments and supplies, which can be attached either to the right or left side of the table, at the convenience of the examiner.

In doing drainage work, the patient is placed on the table on his right side in a reclining position, with the right leg and knee extended, and the left leg and knee flexed, overlapping the right, thus bringing the duodenum lower than the stomach. The drainage outfit is then withdrawn from beneath the table by releasing the screw which holds it in place. The rack is released by another screw, and is drawn upward and the syringe supporter turned outward. As this whole moves up and down and in and out it can be raised to and fixed in the required position. The test tubes are then placed in the perforated section of the rack, the syringe in the concave support, and the preparations are complete for the examiner to commence his work.

When the drainage work is over, the rack can be lowered, the syringe supporter turned inward, the screws refastened, and the entire outfit slid beneath the table.

For rectal work, the special tray is withdrawn and the instruments and supplies placed thereon, within convenient reach of the physician. The work completed, the instruments are removed and the tray slid beneath the table.

ADVANTAGES

- 1 It is a standard table, of dimensions convenient for the examiner.
- 2 The soft cushioned leather pad adds to the comfort of the patient.
- 3 The adjustable headrest permits the head to be raised or lowered as required.
- 4 A complete drainage outfit is attached, and can be arranged in any position desired. This outfit includes the following:
 - a a test tube rack to hold the test tubes securely in place to avoid spilling
 - b a small box to contain the supplies
 - c a concave support that holds the syringe
- 5 The rectal tray, for the necessary instruments, can be hooked on either side of the table, at the convenience of the examiner.

AN IMPROVED STIMULUS SELECTOR*

By RICHARD HOMER FITCH, M A, MADISON, WIS

IN EXPERIMENTATION involving the stimulation of the cortex at variable frequencies and intensities it became necessary to devise a stimulus selector of a type which could be shielded to prevent amplifier pick up and which would give constant frequencies and intensities at the settings utilized. The rotary commutator type of stimulus selector was found to possess several disadvantages for these purposes. The brushes have a tendency to skip, and it is difficult to secure a constant resistance break on the commutator surface, and the variation in line voltages supplying the motor to the rotary selector results in inconstant frequencies and potentials. A type of stimulus selector incorporating an oscillating neon tube as developed in England by Daly,¹ was set up in an attempt to overcome these difficulties. It was found that in this arrange

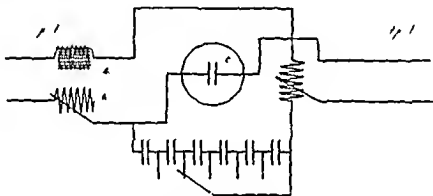


Fig 1—A 0.5 megohm variable grid leak B Iron core choke (secondary of Thorlanson audio transformer) C Neon tube (supplied by QRS Company, Chicago) D Output resistance 10,000 ohms variable F 0.1 MFD condensers in parallel for 250 volt

ment the voltages secured were too low, and the type of neon tube available on the market would not oscillate at the frequencies desired.

The set up as devised is largely empiric, the different values having been arrived at through a more or less trial and error process. A neon tube was made up for us by the QRS Company of Chicago, which was found to be suitable. The commercial television tubes would not operate over a large range of frequencies, due to the fact that the electrodes are not equally spaced. This variation in the distance between them resulted in the entire electrode surface not being involved in the oscillation. The tube furnished by the QRS Company possessed a constant interelectrode distance and was found to operate satisfactorily at frequencies ranging from once in four seconds to one hundred per second. Higher frequencies can be obtained by use of a tube of smaller electrode area. If a very high potential output at high frequencies is desired, it is probably impractical to use this type of stimulus selector as the potential varies inversely with the frequency.

The output circuit, as diagramed in Fig 1, does not supply high enough voltages for stimulating all tissues, the maximum being approximately 15 volts. It can, however, be connected to the primary of a Harvard induction coil which in our experience, gave a maximum potential of 85 volts.

Thinking that the use of the step-up transformer and an iron core choke coil, which was included in the D C supply main, might change the characteristics of the oscillations from those described by Pearson and Anson,² an attempt was made to record the form of the stimulating current by means of the string galvanometer. It was found that a string sufficiently tight to follow the rapid change would continue to vibrate at its own period after the discharge from the selector had ceased giving the impression of a polyphasic fluctuation with decrement. If the string was slackened to its critical aperiodic point to prevent its vibrating, the resulting form was monophasic but was unduly extended in time, the string being unable to follow such a rapid change.

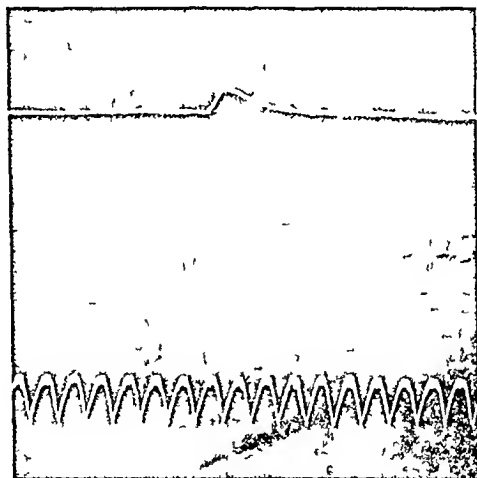


Fig 2—Oscillograph of wave form A Stimulus selector B Time 1/1000 second

Oscillographic pictures were then taken (Fig 2) by means of a Westinghouse oscillograph with a period of approximately 2,000 cycles. The period of rising potential was found to occupy 0.52 sigma, and the total duration of the impulse 2.6 sigma, the form being monophasic.

This form of stimulating current is of particular advantage, because the lower frequencies possess the same rate of potential change as the higher, and consequently have the same stimulating value.

The operation of the set-up simply involves the adjustment of A and E (Fig 1) for varying the frequency, and setting D according to the potential output desired. The high voltage direct current was secured from a radio "B" eliminator but can also be obtained from "B" batteries or motor generator.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE MD, ABSTRACT EDITOR

TISSUE A Short Method of Clearing Plant Tissues Simpson J L Stain Tech 4 131
1929

The material to be studied is placed in lactic acid of a concentration of about 75 per cent. Whole blossoms or large parts such as the pistil may be immersed in the medium in an open watch glass. Sections of fresh material cut fairly thick by hand may be mounted in the acid on a slide and the cover glass applied. In either case the specimen is then placed in a constant temperature oven and kept at about 50° C until clear. The time required varies with the material. Sections of floral parts, young fruits or succulent stems become clear in two or three hours. Whole parts or thick edges of such material have required in the writer's experience about twelve hours. It is often convenient to leave them overnight.

If permanent slides are desired, the lactic acid may be thickened by slow evaporation in the oven until it is almost hard. The edges of the preparation may then be sealed. For this purpose, a mixture of gum mastic and paraffin applied with a hot iron wire, has been found suitable. As lactic acid is hygroscopic the slides become very sticky if left unsealed. In cleaning up such slides it is useful to remember that lactic acid is soluble in alcohol.

CANCER A Serum Test for the Diagnosis of Cancer Based On a New Theory of Etiology
Gruskin, B. Am J M Sc 177 476 1929

Preparation of the amboceptor for carcinoma. Mammalian embryos are used (calves, sheep, pigs). They must not be in a later stage than the second month of pregnancy. This is readily recognized by their relative smallness, and by the smoothness of the skin (for instance, there is no formation of hair). In securing these embryos the abdomen must be opened under sterile conditions. The pancreas and submaxillary glands are dissected out and placed in a sterile dish. A hypotonic salt solution is poured over these and if possible allowed to freeze, the object being to permit an easier removal of the fibrous capsule. The capsule and the ducts etc. are then removed by careful manipulation with small tissue forceps. The epithelial tissue is picked out. It is placed in a mortar in which sterile copper gauze is inserted (to facilitate the maceration) and macerated. Salt solution is added and thoroughly mixed and rubbed up with the macerated tissue until the fluid becomes milky or opalescent in appearance.

The cells suspended in salt solution are centrifuged until the supernatant salt solution is perfectly clear. The salt solution is then discarded and the epithelial cells are placed in a porcelain dish and dried at a temperature of 75° C to a doughy consistency. During this period, it is essential that the tissue be stirred thoroughly at fifteen minute intervals in order to permit uniform drying. The tissue is then placed in a glass stoppered bottle to which is added three times its volume of acetone. The mixture is permitted to stand in the ice chest for twenty four hours with frequent shaking. The acetone is then poured off and replaced by one and a half volume of fresh acetone for another twenty four hours. The acetone is again decanted and the tissue is placed in a mortar to which five times its volume of absolute alcohol is added. This is macerated for about fifteen minutes until the alcohol becomes somewhat milky. The mixture is then kept in the ice box for five days during which time it is forcibly shaken at two hour intervals. The alcohol extract is then decanted and is ready for use.

The output circuit, as diagramed in Fig 1, does not supply high enough voltages for stimulating all tissues, the maximum being approximately 15 volts. It can, however, be connected to the primary of a Harvard induction coil which, in our experience, gave a maximum potential of 85 volts.

Thinking that the use of the step-up transformer and an iron core choke coil, which was included in the D C supply main, might change the characteristics of the oscillations from those described by Pearson and Anson,² an attempt was made to record the form of the stimulating current by means of the string galvanometer. It was found that a string sufficiently tight to follow the rapid change would continue to vibrate at its own period after the discharge from the selector had ceased giving the impression of a polyphasic fluctuation with decrement. If the string was slackened to its critical aperiodic point to prevent its vibrating, the resulting form was monophasic but was unduly extended in time, the string being unable to follow such a rapid change.

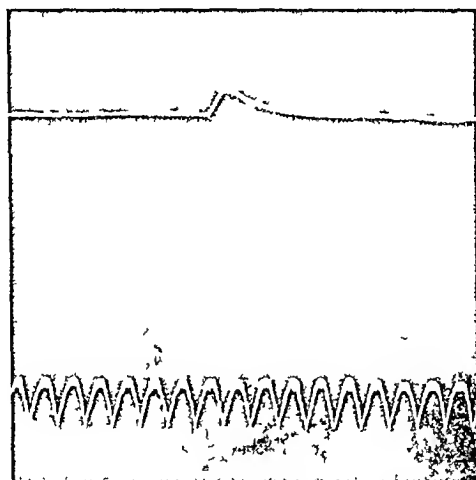


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BLOOD CHEMISTRY Tungstomolybdic Acid as a Precipitant for Blood Proteins Benedict S L and Newton E B J Biol Chem 83 357, 1929

A protein precipitant is described which does not cause the loss of any nonprotein constituents and which thus permits the quantitative recovery of thionine and glutathione from blood. It is prepared and used as follows: 10 gm of pure ammonia free molybdic acid is treated in a flask with 70 cc of NH_4OH and the mixture boiled for one to two minutes. A practically clear solution should result. About 150 cc of H_2O is added and the cooled solution is mixed with a solution of 80 gm sodium tungstate dissolved in about 600 cc H_2O . This mixed solution is diluted to 1 liter. The acid employed during the precipitation is 0.62 N sulphuric acid prepared by diluting 620 cc of N acid to 1 liter. The precipitation of blood proteins is carried out as in the familiar tungstic acid precipitation.

ACETOACETIC ACID IN URINE Simple Determination of Melka J and Klein F Bratislav Lekarske Listy 8 188, 1928

The acetoacetic acid in 5 cc urine is decomposed by the addition of 1 L drops of N sulphuric acid and heating in a water bath for fifteen minutes. After cooling and making alkaline with $NaOH$ ferric chloride solution is added and sufficient 1 per cent solution of iodoacetic acid to give a color reaction of the same intensity as that obtained from the original sample of urine. The amount of acetoacetic acid added is equal to that originally present.

TYPHOID A New Enrichment Method for Typhoid and Paratyphoid Bacilli In Water Hader F Zentr Bakt Parasit 113 353, 1920

A stock solution of malachite green of 1:250 and of brilliant green 1:200 was used. Mix 38.5 cc of sterile water, 5 cc of sterile bile and 0.5 cc of bouillon and add the material to be examined. Add 0.8 cc of stock malachite and 0.1 cc of brilliant green drop by drop. Incubate three hours and add 0.45 cc of malachite and 0.15 cc of brilliant green and continue the incubation. The modification is very satisfactory.

VACCINES A New Method of Producing Bacterial Vaccines Vignati J Zentr Bakt Parasit 113 71 1929

Bacterial suspensions were made in 0 to 10 per cent $CuSO_4$ solutions allowed to stand at room temperature for four to six hours, centrifuged and diluted with the following solution: $Na_2S_2O_8 \cdot 5H_2O$ 25 gm, Na_2SO_4 25 gm, distilled water 1000 cc. These vaccines were just as antigenic and less toxic than heat killed cells.

PNEUMONIA Icterus Index Studies In Lobar Pneumonia, Elton N W N Eng J M 201 611 1929

The following conclusions are advanced:

1. In primary lobar pneumonia a latent jaundice constantly occurs.
2. The icterus index is often found in the range of clinical jaundice yet clinical jaundice may not definitely be present.
3. The icterus index reaches its highest values in cases terminating in true crisis. No fatal case in this series had an index exceeding 17.
4. The latent jaundice usually subsides precipitously upon the establishment of a fluid pleural exudate.
5. The graph of the daily icterus index curve of the cases in this series shows a rising curve occurring in the presence of a purely intravascular pneumonic process until crisis or death, and a declining curve in the presence of a fluid pleural exudate. The latter type of curve was always associated with cases that were potential empyemas.

6 Conclusions based on etiology must be deferred Although the pneumococcus, which is bile soluble, is commonly regarded as the etiologic agent in primary lobar pneumonia, it cannot be stated that it was the causative organism in all the cases of this series which underwent the phenomenon of crisis

CHOLECYSTITIS Bacteriological Study of a Group of Diseased Gall Bladders Branch, C F N Eng J M 201 308, 1929

1 In chronic cholecystitis the gall bladder is infected in approximately 12 per cent of the cases

2 In acute cholecystitis the gall bladder is infected in approximately 75 per cent of the cases

3 Approximately 40 per cent of all gallstones are infected

4 Bile in which the bile salts have a normal or high concentration has a marked inhibitory effect on bacterial growth

5 The predominating organisms recovered from infected gall bladders in the order of frequency are B coli, streptococci and staphylococci

6 The organism recovered from a case of acute cholecystitis is not necessarily the primary etiologic factor responsible for the development of the acute lesion

7 Organisms recovered from the "cystic gland" are not a fair criterion on which to base an assumption of existing infection in the gall bladder

NEGRI BODIES Stain for, Jordan, J H and Heather, A H Stain Tech 4 121, 1929

Fixation

1 Zenker's solution four hours at 37° C or Dominici's three hours

2 70 per cent alcohol, twelve to eighteen hours at room temperature

3 80 per cent alcohol, about five to six hours

4 90 per cent alcohol, about four to six hours

5 Absolute alcohol about sixteen hours

6 Ether and absolute alcohol aa, about eight hours

7 Sixteen to twenty four hours in the following mixture celloidin 1 g, methyl salicylate 25 cc, absolute alcohol 25 cc, ether 25 cc

8 Chloroform and paraffin, two to three hours

9 Paraffin, two hours

10 Paraffin, one to one and one half hours

11 Embed

Staining

1 Cut sections 4 to 5 micron

2 Bring section to water and cover with Lugol's iodine for ten minutes

3 Decolorize with a 2 per cent sodium thiosulphate (hypo)

4 Wash thoroughly with water

5 Cover with a mixture of equal parts of 0.5 per cent phloxine and 1 per cent eosin Y (National Aniline brand) and leave for fifteen minutes

6 Wash with water and stain two to five minutes in 0.1 per cent azure B (National Aniline)

7 Wash with 96 per cent alcohol and decolorize in a mixture of 2 parts absolute alcohol with 1 part clove oil, ordinarily for not more than one half to one minute

8 Dehydrate rapidly, clear, and mount in Yucatan Eleni

OCCULT BLOOD A Modification of The Strzyzowski Reaction Oustinoff P V Ann
do Med Leg 9 477, 1929

Glacial acetic acid	1 cc
Gum arabic (25 per cent)	1 cc
or	
Glycerin	1 cc
Hydrotic acid	2 to 3 drops

This reagent can be kept for two to three weeks

A small fragment of the suspected substance which should be entirely dried is placed on a glass slide and is covered with a cover glass. A few drops of the reagent are then added. The preparation is heated until it boils for ten to twenty seconds more reagent should be added. The crystals thus formed are small and are rhombic in shape and are black prisms of hematine iodide.

ALBUMIN A New Test for Rose C Indian M Gaz 64 17 1929

The reagent used for the new test is a saturated solution of saccharin in water prepared by boiling saccharin in distilled water until no more saccharin is dissolved. Allowing the solution to cool the next step is to filter it and preserve the clear solution in a stoppered phial. On standing for some time a few crystals of saccharin may separate out and settle at the bottom these may be allowed to remain.

The test may be performed in the same way as one does Heller's test with nitric acid. It is best to take a long and narrow test tube about one sixth full of clear urine and hold in a slanting position. Now slowly and carefully allow the reagent to run along the side of the test tube by means of a pipette it will settle at the bottom. If albumin is present in the urine even in traces a sharply defined white ring will slowly form at the junction of the two layers of fluids best seen when held against a dark background. The ring does not disappear on the application of heat. Strong nitric and picric acids behave in a similar way with albumin in the urine and with regard to delicacy all tests are probably of equal merit but there are certain advantages which the saturated solution of saccharin possesses over the other tests.

The new reagent (saccharin) possesses several advantages over nitric acid in that

- 1 It does not precipitate mucin
- 2 It does not precipitate urea
- 3 It does not precipitate urates unless these are present in the urine in considerable excess

WASSERMANN REACTION As a Routine Test In Hospital Practice Nickson D H
and Leibly F J Arch Dermat & Syph 19 738 1929

In a total of 17 132 consecutive Wassermann tests on adults the authors found an average of 19 per cent of the hospital patients to be syphilitic. The diagnosis was made by the Wassermann test in 248 or 74.2 per cent of the 334 cases leaving 25.8 per cent in which the diagnosis was made by the physician or surgeon in charge before the Wassermann test was done.

From the foregoing facts it may be concluded that

- 1 Routine Wassermann reactions in hospitals are not only justifiable but necessary for the diagnosis in three of every four syphilitic patients admitted
- 2 The percentage of syphilitic patients admitted to the Swedish Hospital has been found to be considerably less than that in other general hospitals
- 3 Syphilitic men present themselves in hospitals in a two to one ratio compared to women
- 4 Women in hospitals show the presence of syphilis on an average ten years earlier than men

5 It is approximately 10 per cent easier to diagnose syphilis in men than in women on a basis of history and physical observations

6 The number of unmarried men with syphilis is high compared to the number of married men who have it, and an unmarried man of middle age is more likely to be syphilitic than a married man of the same age

7 The mortality rate for syphilitic infants in this hospital was 40 per cent

FLAGELLA A Method for Staining, Craigie, J Brit J Exper Path 9 55, 1928

Suspensions of twenty four hour cultures are fixed in formal (physiological saline, or buffer solution at P_H 7, containing 2 per cent formalin) Leave in fixative one hour or overnight Glassware is cleaned in bichromate sulphuric acid Dilute formalized suspensions in distilled water and spread a small loopful over a cleaned slide Dry at $37^\circ C$ Heat at 90 to 100° for five minutes or longer Place in distilled water five minutes, then wash in distilled water Dry and heat again at 90 to 100° *Mordanting* Use Zettnow's mordant (10 gm tannic acid, 200 cc distilled water, heated to about 60° and 30 cc of 5 per cent aqueous tartar emetic added slowly) Flood slide with mordant and heat five to ten minutes at 90 to $100^\circ F$, watch constantly to prevent drying at edges by adding more mordant as required Wash under tap, then in distilled water *Silvering* Dissolve 1 gm silver sulphate in 250 cc distilled water, and to 20 cc of the solution add mono ethylamine solution (33 per cent B D H) until opacity just clears up Flood slide with this and warm till steaming begins, holding at mild heat till preparation turns brown or black, but no longer Wash under tap, then in distilled water *Gold toning* Add 20 drops of 1 per cent gold chloride solution to 20 cc distilled water Immerse slides in this for thirty minutes or longer Wash and dry

TUBERCLE BACILLUS Rapid Staining of, Gaussen, C Compt rend Soc Biol 97 1657, 1927

This method stains Koch's bacillus, other organisms, and cells in sputum and is useful for pathologic serous exudates, cephalic spinal fluid and urine Make thin film on slide dry at 37° , fix in ether alcohol, place on hot plate and stain five minutes in Ziehl's solution, made up by triturating in a mortar 1 gr basic fuchsin, 5 gr phenol, and 10 cc 95 per cent alcohol, add 90 cc distilled water and filter After staining wash in running water, dry, stain for four minutes in a differentiating stain made up as follows

Dissolve separately (warm) in round bottom flasks

1 Two and five tenths gm Methylene blue, 150 cc 80 per cent alcohol, 3 gm lactic acid,

2 Two gm orange G, 100 cc 80 per cent alcohol, 2 gm lactic acid,

Put aside 50 cc of the blue solution to cool Mix the rest of blue with orange, heat again, cool, filter Heat precipitate remaining on filter with 30 cc 80 per cent alcohol Cool, filter, mix two filtrates and add the 50 cc of blue solution that has been put aside, filter

Nuclei are stained violet, acidophil protoplasm yellow, basophilic blue eosinophil granulations greenish, mucus violet, sero albuminous exudates yellow, microorganisms blue green against yellow cytoplasm, Spirillae are gray, diplococci deep violet red, common acid resisting organisms deep maroon Koch bacillus bright red

TISSUE STAIN Preparation of Muci Carmine, Southgate, H W J Path & Bact 30 729, 1927

Precise directions for preparing Mayer's mucicarmine which give constant results are given One gram of carmine, 1 gr of dry powdered aluminum hydroxide 100 cc of 50 per cent alcohol, 0.5 gm of powdered anhydrous aluminum chloride are placed in a 500 cc flask which is placed on a boiling water bath with frequent shaking The mixture is boiled exactly two and one half minutes, cooled under the tap and filtered For staining dilute

1 cc of this solution with 9 cc of distilled water and stain sections fifteen to twenty minutes. Rinse with distilled water then treat with alcohol xylol and balsam. This stain colors mucin deep.

CARCINOMA Rapid Histologic Diagnosis Dengler R. Zentralbl f Gynak Leipzig 53 457 1929

A piece of the suspected tissue is removed with a pair of fine forceps or a sharp instrument and placed in one drop of 0.9 per cent sodium chloride solution on a slide. It is then torn into shreds with two dissecting needles, covered with a cover glass and examined under a microscope. After cutting down the light and turning to the highest magnification one determines whether or not the preparation is sufficiently transparent. It is a drop of 1 per cent acetic acid is placed at one edge of the cover glass. In a short time the microscopic picture becomes clear. The nuclei become distinctly visible particularly if the diaphragm is closed still more. If one finds in the preparation an area which is sufficiently transparent and which contains almost isolated cells lying side by side one may begin the counting. The author divides the cells into seven classes according to the size of their nuclei ranging from Class 1 with the smallest nuclei to Class 7 with the largest nuclei. Each higher class consists of cells whose nuclei are two three four five six or seven times larger than the smallest nuclei in the tissue. This simplified technique is possible because the size of the nucleus may be taken as an index of the malignancy of a cell. A differential nuclear count of several fields containing the smallest and the largest nuclei should be made in order to get a truly representative count. Erythrocytes, lymphocytes, phagocytes, leucocytes, fibroblasts, and giant cells are readily recognized and are not counted. The result of the count is expressed by the quotients of the count for the various size classes by the total numbers of cells counted. When fifty cells are counted it lies between 3/50 and 7/50 or 0.06 and 0.14. In cancer it is always more than 0.1 usually 0.14. In all of 100 cases in which this method of diagnosis was used the results were confirmed by the histologic examination of the specimen removed at operation.

SPUTUM STUDIES IN CHILDREN Direct Laryngoscopy As a Method for Cultural Studies of Pulmonary Secretions In Infants and Children Goldman I B. Am J Dis Child 38 47 1929

Attention is called to the utilization of the direct laryngoscope for obtaining for cultural and other examinations sputum or laryngeal and tracheal secretions from infants and children.

SICKLE CELL ANEMIA In a Greek Family Cooley T B and Lee P. Am J Dis Child 38 103 1929

The authors report the occurrence of this condition in a Greek family, thus indicating that it is not as has been thought peculiar to the negro race. They suggest that sickle cells should be looked for in all hemolytic anemias.

OTITIS MEDIA In Infancy Diagnosis By Means of Cultures Taken From the Middle Ear O'Donnell W S and Myers C. Am J Dis Child 38 49 1929

The contents of the middle ear are obtained by means of a specially made needle 19 gauge $3\frac{1}{2}$ inches (8.9 cm.) long. To the needle a glass observation tube (glass window) is added. A piece of soft rubber tubing 3 inches (7.6 cm.) long is placed between the observation tube and a glass mouthpiece. With this apparatus the contents of the middle ear can be withdrawn by aspiration. Before the apparatus is used it is sterilized by autoclaving in a test tube with a cotton stopper.

The child is wrapped in a blanket and placed on an examining table. The head is held quiet by an assistant. The cerumen and the contents of the aural canal are carefully

removed. Absolute alcohol is then dropped into the ear. Next, sterile cotton is inserted and removed after five minutes. A sterile speculum attached to an electric auriscope is placed in the ear. The needle is inserted into the speculum and through the drum. By suction with the mouth, the contents of the middle ear are withdrawn into the observation tube. The material obtained is immediately transferred to a slide of sterile physiologic sodium chloride solution in a small test tube.

Cultures are made from the suspension in physiologic sodium chloride solution. The material is placed in each of two tubes of veal infusion pneumococcus broth, P_H 7.4. In addition streaks are made with a platinum wire on a beef extract, agar blood plate, P_H 7.8. At the same time, a smear of the suspension is made, and examined with gram's stain.

Observations are made before the needle is inserted as to the appearance of the drum, its color and the amount and position of the bulging. This is done in order to ascertain whether the organism cultured from the contents of the middle ear is associated with any characteristic changes in the tympanic membrane.

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan Medical Arts Building,
Richmond, Va

*Biological Stains*¹

PROBABLY few volumes of similar size will prove more useful to the laboratory worker than this present work.

The outgrowth of the work done by the Commission for the Standardization of Biological Stains this new edition much enlarged and comprehensively reviewed, contains a wealth of information. Many of the outstanding staining methods are presented together with numerous useful tables.

The reviewer knows of no other source where this extremely useful information can be found without an exhaustive search of the literature.

The Nervous Child[†]

THE successive editions of this book have always been welcome, especially to pediatricians and internists, and this fourth edition has been further enhanced by an additional chapter on the "Underlying Disturbances of Metabolism in the Nervous Child" which deals with that more or less baffling group of infants and children who possess that vague and indefinable background referred to by the German school as *Exudativo Diathesis*. This class embodies eczema tetany cramp convulsions urticaria and asthma and no study of a nervous child can be considered complete which does not attempt an analysis of this aspect of the problem. This grouping is usually designated by British writers as the "lymphatic type," while the antithesis is the "acid type."

This book can be recommended to that particular class of parents who are the sometimes proud possessors of the negative type of child, and especially the type of infant or child with a neuropathic background. Of extreme value and practicability is the chapter dealing with poor appetite which the author portrays skillfully and intelligently as one of the perversities of the recalcitrant eater. Marked emphasis is laid upon environment and surcharged atmosphere 'healthy inattention' and stubbornness greater than that possessed by the child are some of the happy and successful relations offered.

This small book of 16 chapters with illustrations consists of 236 pages is clearly and concisely written in an interesting manner, almost novel like in character, as that great pediatric clinician, Dr Cameron, can portray. This book since its first edition in 1910 is constantly quoted as embodying and exemplifying the practical processes successfully proved by years of experience. This small volume should find a place on every physician's desk as a medium to aid in imparting proper understanding and suggestions to parents, especially those with obstreperous offspring. There are a number of excellent photographs depicting 'types' of children particularly emphasizing the "status catarrhalis" and 'postural defective'. It would be well worth Dr Cameron's efforts to present such a book for lay reading.

Biological Stain. A Handbook on the Nature and Uses of the Dyes Employed in the Biological Laboratory. By H. J. Conn. Second Edition. Cloth. 224 pages. The Commission on Standardization of Biological Stains. Geneva, N. Y.

[†]**The Nervous Child.** By H. C. Cameron. M.A. M.D. (Cantab) F.R.C.P. (Lond.) Physician in-charge of the Children's Dept. Guy's Hospital. Fourth Edition. Oxford Press.

NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

Outline of Bacteriology

THIS small volume is practically a quiz compend for the use of students of oral hygiene, and embodies the material presented by the author to his classes in Columbia University

Elementary in character, it should prove useful to those to whom it is addressed

Fundamentals of Pathology†

THIS little volume is intended primarily for dentists and students of oral hygiene and is based upon lectures delivered by the author in the School of Oral and Dental Surgery, Columbia University

It is, of necessity, succinct and compact and as such constitutes an effective quiz compend very useful to those to whom it is addressed

Memoranda of Toxicology‡

THE second edition of this well known little handbook bears evidence of comprehensive revision with the inclusion of much new material concerned not only with newer methods for the treatment of poisoning, but also the newer sources of poisoning, such as tetraethyl lead, barium salts, and asphyxiating gases

The book contains a wealth of authoritative material within a small compass and should be exceedingly useful to the student and practitioner

Diseases Transmitted from Animals to Man§

THAT disease may be interchanged between man and animals is a matter of general knowledge, but exact information as to *what* diseases, and *exactly* how they are transmitted as well as the effective means for the prevention and control of such infections is not as generally disseminated as it might be possibly because this information is to be found in numerous and sometimes out of the way places

In compiling this book, therefore, the author has done no small service to the clinician, the epidemiologist, and the pathologist

The book is divided into 5 sections

Section I covers Diseases of Domestic Animals and Birds, Section II, Rodent Affections, Section III, Human Diseases Spread by Animals, Section IV, Animals as Passive Carriers of Disease Organisms, and Section V is devoted to a review of the Rôle Played by Each Animal in the Spread of Disease

There is also an alphabetical index of authors quoted or referred to in the text

At the end of each chapter the salient features of the disease in question are reviewed in a series of short, crisp paragraphs

The book is a useful and valuable addition to medical literature deserving of wide circulation

It can be highly commended to all who are interested in the problem of disease

*Outline of Bacteriology By H. A. Bartels Lecturer on Bacteriology School of Oral Hygiene Columbia University Cloth 128 pages 48 figures W. A. Broder New York

†Fundamentals of Pathology By Joseph Schroff Assoc. Prof. Oral Pathology Columbia University Cloth 119 pages 40 figures W. A. Broder New York

‡Memoranda of Toxicology By M. Truffer Consulting Toxicologist Graduate School Univ. of Penna. Second edition Leather 214 pages P. Blakiston's Son & Co. Philadelphia

§Diseases Transmitted from Animals to Man By T. G. Hull Chief Bacteriologist Illinois Dept. Health etc. Cloth 350 pages 20 illustrations C. C. Thomas Springfield Ill.

Recent Advances in Preventive Medicine¹

PREVENTIVE medicine no longer means merely what is now called sanitation but embraces not only every field of medicine but sociologic and physiologic aspects as well.

In the present volume is given a fairly comprehensive and diverse survey of the recent advances in this field.

Among the subjects considered are eugenics maternal mortality wastage of young life childhood milk and milk borne disease recent advances in our knowledge of vitamins atmospheric conditions hygiene in industry and active immunization.

The discussion of all of these subjects is drawn from a thorough survey of the literature, a list of the references utilized being appended to each chapter.

The volume should be of interest and value to all who are concerned with the problem of disease.

Human Helminthology†

THERE are few subjects in medicine concerning which information is less generally disseminated than helminthology possibly because of the irregular geographic distribution of the diseases in which it is concerned and perhaps because of the special training required for its competent study.

In this present volume Dr Faust, whose experience well qualifies him for the task presents a comprehensive text embodying all the important steps in its recent developments.

Section I of the book covers chiefly the fundamentals of the subject including the literature. Section II discusses systematically the flatworms and Section III the roundworms.

Section IV is of great interest and value as it presents the laboratory problems concerned with the study of helminthology.

The newer methods are clearly described and when necessary amply illustrated. Chapter XXVII The Identification and Differential Diagnosis of Helminth Parasites and Their Eggs will prove exceedingly useful in those laboratories where this kind of work is relatively infrequently done.

The final chapter concerns the intermediate and reservoir hosts involved.

All in all the volume is a credit to both author and publisher and seems destined to become a standard text. It can be highly recommended to both physician and laboratory worker.

Tularemia‡

THE gradual recognition of tularemia as an endemic infection of marked economic importance makes this volume most timely.

Its history pathology, diagnosis, and treatment are comprehensively discussed by Dr Simpson whose work in connection with the disease is well known.

In view of the fact that many cases have been overlooked in the past the chapter on the clinical manifestations of tularemia will be most useful.

The illustrations, which are numerous are excellent in every way.

The volume can be recommended without reserve and should be in the hands of every practitioner.

Recent Advances in Preventive Medicine. By J. F. C. Henshaw, Assistant Director, Bureau of Hygiene and Tropical Diseases, etc. With A Chapter On the Vitamin by S. J. Cowell. Professor of Dietetics, Univ. of London. Cloth 328 pages 30 illustrations. P. Blackiston's Son & Co. Philadelphia.

Human Helminthology. A Manual for Clinicians, Sanitarians and Medical Zoologists. By Ernest Carroll Faust, Ph.D. Professor of Parasitology in the College of Medicine of Tulane University, New Orleans, La. Octavo 616 pages illustrated with 297 engravings cloth. Lea and Febiger Philadelphia.

Tularemia: History, Pathology, Diagnosis and Treatment. By W. M. Simpson. Cloth 162 pages 53 figures 2 colored plates. P. B. Hoeber New York.

Arthritis^{*}

CHRONIC nontuberculous arthritis is receiving considerable attention. Literature upon this subject is rapidly accumulating. Obviously, it is becoming more and more difficult for the practitioner to choose his reading material from the recent volumes on the subject. The average physician has not time to speculate upon theory. He must have his feet firmly set upon the foundation of basic principles.

Fisher has built his book upon a clear insight into joint physiology, and has elaborated his contribution along physiologic lines. His treatment of joint pathology is somewhat different from that of similar works. The effort has been made to give more space to description of early joint changes without neglecting the usual classical pictures of advanced pathology. This tends to clear the misunderstanding that has arisen between the pathologist who sees only terminal changes and the clinician who most frequently attacks the disease in its incipency.

Therapy is adequately treated under three classifications, first, medical treatment, second, physiotherapy, manipulation, and other orthopedic measures, and third, surgical operations in chronic arthritis.

The volume is profusely illustrated.

Wiggers' Electrocardiography[†]

WHEN Wiggers writes on the heart one expects it to be good. As usual this time it is. The volume is based upon the author's lectures to his students at Western Reserve University on the heart and particularly upon the use of the electrograph and the interpretation of the electrocardiogram. The work is divided into three sections. The first section deals with apparatus, technique, the physics of electrocardiography, and describes and discusses the various instruments on the market. The author preserves a strict impartiality as to the merits of this or that machine.

The second section describes and discusses the normal electrocardiogram and the significance of normal and abnormal deflections as observed in clinical work.

The third section is decidedly an innovation and is the portion of the book that makes it different from other volumes on electrocardiography. In it the author presents a series of abnormal tracings as unknowns and proceeds forthwith to analyze each tracing ending up each time with a diagnosis, discussion and rather comprehensive review of treatment. It reminds one somewhat of Cabot's Case Reports which have become so deservedly well known.

The unknowns have been selected and arranged so as to cover all of the usual and most of the unusual types of tracing that one is likely to encounter. In general there is only one example of each. While the discussion is consequently complete we nevertheless hope and anticipate that in the next edition Dr. Wiggers will include a large number of additional unknowns with notes on their interpretation so that the student may have further opportunity for developing his skill in interpretation.

The volume is primarily a textbook for students and a reference work for the internist, who wishes to acquire reasonable skill in electrocardiographic interpretation. As such the reviewer feels that it is the best work on the subject that has come out since Lewis' *Clinical Electrocardiography*.

The Immunology of Parasitic Infections[‡]

THE author has undertaken to compile the scattered observations on this subject, the literature of which is surprisingly large. As a consequence the volume will be of great interest to those interested in clinical and experimental serology.

*Chronic (Nontuberculous) Arthritis. By A. G. Imbrell Fisher, M.C., F.R.C.S. (Eng.) Joint Lecturer on Operative Surgery, London School of Clinical Medicine. Cloth, 186 illustrations, pages 220. The Macmillan Company, New York, 1929.

†Principles and Practice of Electrocardiography. By Carl J. Wiggers, M.D., Professor of Physiology in the School of Medicine of Western Reserve University, Cleveland, Ohio. With 61 illustrations. Cloth, pages 226. The C. V. Mosby Company, St. Louis, 1929.

‡The Immunology of Parasitic Infections. By William H. Tullaferro, Ph.D., Professor of Parasitology, The University of Chicago. Cloth, 414 pages. The Century Company, New York, London, 1929.

It will be of interest to those who have not made a special study of the subject, to know that there is a fairly promising flocculation reaction for kala azar and a reliable complement fixation reaction for horse trypanosomiasis. There is evidence of antibody formation in anilaria but so far no serologic test has been perfected to the point where it can be of practical diagnostic aid. The hindrance in preparing a suitable test antigen lies in obtaining sufficient malaria organisms free from host protein. So far there is no satisfactory serologic test for coccidiosis. The results in schistosomiasis are promising but so far inconclusive. In hydatid disease the intradermal test for echinococcus infection is very promising. There is promise of a satisfactory precipitin test for trichinosis after infection has been well established and for an intradermal test for early infection. Although in no way standardized there seems considerable promise that serologic tests may be developed in amebiasis. The Wassermann test for syphilis, with the more modern methods is not positive in various parasitic infections of man unless syphilis or yaws is present. On the other hand it may be positive with the serums of rabbits infected with trypanosomes.

There is evidence that in experimental trypanosomiasis the animal develops a trypanolytic immunity which increases and decreases periodically in the infected animal. The serum of animals which have recovered from this disease appears to carry immune bodies which may be used for passive immunization.

Eosinophilia is characteristic of many of the protozoan invasions. Eosinophilotactic substances have been obtained from many worms and our efficacy in producing eosinophilia is largely dependent upon previous sensitization. The reticuloendothelial system, presumably the source of immune bodies, is known to be parasitized by the Leishmania and certain of the sporozoa which by their invasion appear to inhibit or decrease antibody production. However the reticulo endothelial system is the chief defense of the body in malaria, is responsible for the formation of the humoral antibody which inhibits cell division of *T. Lewis*, and it is important in the activation of the trypanocidal property of normal human serum and of drugs.

The author makes one very pertinent statement which we cannot refrain from quoting: 'There is a tendency to treat antibodies as if they were definitely known chemical complexes when as a matter of fact, they are known only as properties or manifestations of antiserum and are postulated only in terms of what the antisera will do under certain conditions just as enzymes are known by what they do rather than by what they are.'

*Mammalian Physiology*¹

A BEAUTIFULLY produced laboratory manual for the use of students in experimental physiology well illustrated with kymographic tracings, line drawings and color sketches. Marginal headings facilitate orientation and annotations at the end of each of the twenty-two exercises provides discussion and historical references.

This volume should be in the hands of every teacher of physiology. Some will probably use it as a laboratory manual.

Practical Psychology and Psychiatry[†] [E.]

A TEXTBOOK for nurses and medical students and reference manual for physicians. The first portion touches the high lights of psychology and the second portion presents an excellent recapitulation of clinical psychiatry. The average physician while not a psychiatrist should have a more intimate understanding of diseases of the psyche than he usually does. This work will enable him to brush up on the high lights without going into unnecessary detail. While the psychiatrist would find it rather elementary we can recommend it most highly for the purposes for which it was intended.

Mammalian Physiology. A Course of Practical Exercises. A New Edition. By E. G. T. Liddell, D. M., Fellow of Trinity College, Oxford, and Sir Charles Sherrington, O.M., M.D., D.Sc. (Cantab.), F.R.S. Wynnflete Professor of Physiology in the University of Oxford. Cloth, pages 162. Oxford University Press American Branch, New York, 1923.

[†]*Practical Psychology and Psychiatry. For Use in Training Schools for Attendants and Nurses and in Medical Classes and as a Ready Reference for the Practitioner.* By C. B. Burr, M.D. Sixth edition revised and enlarged with illustrations, pages 38. cloth. Davis Philadelphia.

Bronchial Asthma¹

NOW that developments in the field of allergy have awakened interest in the possibility of relieving bronchial asthma and allied conditions the monographic literature on this subject is becoming more abundant. The greater part of these discussions are based on considerations of protein sensitization.

Alexander has gone back to fundamentals. His work might be termed briefly the structural and functional pathology of bronchial asthma. After a short historic review he presents in detail the known facts of the anatomy and innervation of the bronchi and air sacs. This is followed by a review of the theories of the mechanism of the asthmatic paroxysm and the factors which initiate an attack. The next chapter deals with the pathology of asthma, and the following with its immunologic aspects. There follow chapters on the clinical features, complications, treatment, and prophylaxis. The author's discussion of the effect of asthma on the heart and circulation is especially interesting. The evidence today would indicate that the asthmatic paroxysm itself except for a temporary partial asphyxia has little or no damaging effect upon the heart. Instead of causing cardiac dilatation, if anything the heart is smaller during the attack. Even at postmortem in cases of long standing asthma and emphysema no constant cardiac lesion is to be found.

On the other hand, in chronic pulmonary emphysema there develops some hypertrophy and dilatation of the right ventricle. This presumably is the mechanical result of increased pressure in the pulmonary artery due to partial obstruction in the pulmonary circuit. Emphysema accompanying bronchial asthma does not appear to be associated with this type of structural change.

On the other hand the peripheral venous pressure is markedly increased in the asthmatic attack. It is possible that this partial venous stasis, especially when associated with emphysema, may account for the subcutaneous edema occasionally seen in advanced cases of bronchial asthma. This edema together with the dyspnea upon exertion and the cyanosis resulting from emphysema simulates the picture of cardiac decompensation and has given rise in the past to the misconception that bronchial asthma is likely to produce congestive heart failure.

Alexander stresses the value of sputum examination. Too often nowadays this is overlooked, and yet it may contain valuable information.

In a short appendix the skin tests are described and discussed.

Alexander's monograph covers a field of the subject of asthma which is rather neglected in most of the other recent monographs. It should be in the hands of those who are interested in the subject.

Goiter Prevention and Thyroid Protection†

A SEMIPOPULAR presentation of diseases of the thyroid gland, which presupposes no previous acquaintanceship with the subject on the part of the reader. Like many such discussions of a single subject it places perhaps a little too much emphasis on the thyroid to the exclusion of the rest of the body. It is a question whether some harm is not done by placing this type of book in the hands of the laity, for lay persons who read these works are usually nervous, introspective and who possess no stabilizing background of general medical perspective.

For the physician who has never made any special study of the diseases of the thyroid gland and who wants to have an easily assimilable work on the subject this volume should be of some interest.

¹Bronchial Asthma. Its Diagnosis and Treatment. By Harry L. Alexander. A. B. M. D. Associate Professor of Medicine in the Washington University Medical School. St. Louis, Mo. Associate Physician to the Barnes Hospital. St. Louis, Mo. Cloth pages 171. Illustrated. Lea & Febiger. Philadelphia. 1923.

†Goiter Prevention and Thyroid Protection. By Israel Bram. M. D. Author of Goiter, Nonsurgical Types and Treatment. Medical Director, Bram Goiter Institute, Upland, Pa. formerly Instructor in Clinical Medicine, Jefferson Medical College, Philadelphia. Fellow of the American Medical Association, the Association for the Study of Internal Secretions, the American Association for the Study of Goiter, the American Medical Editors' Association, etc. Illustrated. cloth pages 327. F. A. Davis Company, Philadelphia. 1923.

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EDITORIALS

Barbital Poisoning

ONE of the more common types of poisoning is that with diethylbarbituric acid (veronal). This drug is pretty generally known to the laity and many cases of habit formation from its use have been reported. The acute symptoms are fairly characteristic but may be confused with those of other hypnotics, particularly those of the barbituric acid series. The pupils are constricted (as also often occurs in acute chloral poisoning and to a less extent with codeine) and may thus lead the physician to suspect morphine poisoning.

Much work both clinical and experimental has been done in the past in the matter of investigating and treating cases of acute barbital poisoning. The smallest fatal dose for man has been found to vary between 0.6 and 10 gm, but the average fatal dose for man is probably about 50 gm (3.3 gm). This wide variation in the size of the fatal dose has been emphasized in many clinical reports but the explanation of this phenomenon has been somewhat obscure. Consequently it has been exceedingly difficult to properly evaluate the results of any given form of medical treatment.

In a splendid paper recently published by Gower and Tatum¹ much new light has been thrown on the variations to be found in veronal poisoning. Over a period of two years these investigators have carried on experiments under a variety of conditions and they have found that in carefully controlled experiments most dogs recover from a dose of from 200 to 250 mg per kilogram of body weight given intravenously. On account of its solubility they have used the sodium salt of veronal (medinal). Doses of 400 mg per kilogram were fatal within one to five days in about 70 per cent of the cases. Animals which recover from a dose of 400 mg per kilogram are likely to recover again if the same dose be repeated at a later date. One such animal recovered from this dose on five occasions under various treatments. On the other hand 200 mg per kilogram produced death in some cases. Similar variations among animals, not only of the same but of different species as well, have also been noted by Nielsen, Higgins and Spruth² and by Eddy.³ It is found at autopsy that there is a considerable variation in the amount of the drug present in the different organs and tissues of the body, thus indicating a selective absorption. For example, the central nervous system and the red corpuscles both contain relatively high concentrations of the drug in comparison with other tissues.

Excretion occurs almost entirely by the kidneys. It seems probable that some portion of the drug is destroyed in the body for only about 50 to 90 per cent can be obtained from the urine. The excretion is a slow process and this is of especial importance from a clinical standpoint. The cause of death appears to be respiratory failure, or effects secondary to prolonged coma such as bronchopneumonia, impairment of the circulation with pulmonary edema and cyanosis, lack of proper nourishment, etc. Jacoby and Roemer⁴ believed that veronal possessed a specific toxic action on the smaller blood vessels causing capillary dilatation and a fall of blood pressure similar to that under acute arsenic poisoning. The heart becomes weakened, there is a fall in temperature, the reflexes are sometimes increased and very rarely there may be convulsions, the respiration becomes slow and shallow, the urine is diminished or totally suppressed and necrosis of the kidney epithelium has been described. Dermatitis not infrequently occurs and the pupils may often show rhythmic variations.

The treatment of barbitol poisoning in the past has included supporting measures such as heat, liquid food, heart stimulants and removal of the unabsorbed drug from the gastrointestinal tract. Fluids and drugs have been given to stimulate renal activity and bleeding or the removal of cerebrospinal fluid has been advised. Stimulants have been given to overcome the depression. Clinically it is difficult to determine which of these measures is more likely to be of most help to the patient because of the marked variations in resistance to the poison which different patients may exhibit. But from the experimental evidence Gower and Tatum believe that the surest means of helping the patient is to secure as marked and as prolonged an increase in urinary secretion as possible. For these investigators found that, in dogs, tolerant animals were those that had a high rate of urinary excretion of the drug, while animals that remained in a critical condition for several

days after relatively small doses and recovered slowly were animals possessing a low rate of renal elimination of the drug. They, therefore, believe that tolerance for barbitol is dependent directly on the relative ability of the animal (or man) to excrete the drug in the urine. They found, however, in one of their experiments, that when diuretic procedures (rapid injection of glucose solution) were employed, the fluid of the urine might increase twenty times but that the rate of excretion of the veronal was not even so much as doubled. For while a high urinary output tends to increase the amount of drug eliminated for a time, in the course of an eight or ten hour period the rate falls off so that the total drug output is no greater than would be secured by a lower volume secretion of urine.

The solubility of barbitol in water is limited, however, so that the water volume should exceed that required to maintain the barbitol in solution.

With reference to bleeding the authors conclude that the actual quantity of barbitol removed in withdrawn blood is of no practical significance in comparison to a high rate of renal excretion. Bleeding without return of fluid to the circulation uniformly causes a drop in the rate of urine flow and of barbitol excretion but this drop in excretion may be avoided if the volume of the withdrawn blood is immediately replaced by the injection of physiologic salt solution. In dogs saline infusion following bleeding seemed less likely to cause retention of fluid in the tissues with resulting edema than an infusion alone without the preparatory blood withdrawal. But these measures are scarcely to be recommended and the chief aim of therapy in acute barbitol poisoning should be directed toward the maintenance of optimum renal excretion over a long period of time, and more can be hoped for from consistent moderate diuresis than from extreme diuretic measures. It is desirable that the urine be alkaline in order to favor the solubility of the barbitol.

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In regard to the work of the Research Committee on the problem of Undulant Fever, Dr A S Giordano has kindly offered to furnish the material for doing the skin tests put up in vials for distribution to the Members of the American Society of Clinical Pathologists throughout the country. We would like very much to have as many Members as possible obtain this material, make the tests and report the results to Dr A G Foord, Chairman of the Research Committee, Buffalo General Hospital, Buffalo, New York.

To secure this material, communicate with Dr A S Giordano, South Bend Medical Laboratory, South Bend, Indiana.

At the Ninth Annual Convention in Detroit this June, the American Society of Clinical Pathologists will have more space available for Scientific Exhibits than ever before. We are therefore anxious to have as many of our Fellows as possible present exhibits of interest to the Membership. This is fast becoming a very valuable feature of our Conventions and to stimulate participation in this event there are offered two prizes consisting of a Gold and a Silver Medal to be awarded to the two Members of the American Society of Clinical Pathologists presenting the best scientific exhibit. You may communicate with the Secretary, 256 Metropolitan Building, Denver, Colorado, or Dr C I Owen, Grace Hospital, Detroit, Michigan, to reserve space for this exhibit.

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CLINICAL AND EXPERIMENTAL

THE EFFECT OF COLITIS ON GASTRIC DIGESTION*

BY JAMES I. FARRELL, M.D., CLEVELAND, OHIO

I THE EFFECT OF COLITIS ON THE GASTRIC EMPTYING TIME

INHIBITION of the motility of the stomach on stimulation of the intestine has been observed by a number of investigators Bayliss and Starling,⁴ Cannon and Murphy,⁵ Brunemeier and Carlson,⁶ and Percy and Van Liere,¹⁰ who have found that distension of the colon would inhibit the motility of the empty or digesting stomach the degree of inhibition depending on the condition of the animal and the intensity and duration of the stimulation of the colon. White² has produced colitis experimentally in animals and has observed that a marked irritation of the colon caused a definite delay in the emptying of the stomach, and that moderate or mild irritation had no effect. A number of clinical observations on the relation of the colon to gastric motility have been reported. White²³ studying patients with colitis found that when the cecum is irritable, the stomach empties rapidly. Eisen,²¹ however, examined a large number of patients with appendicitis and found a large gastric residue at the end of six hours. Smithies²⁰ found increased gastric motility in some of his cases of colitis. White²³ found that in patients with marked colitis gastric emptying was delayed. Carman and Moore⁹ reported that in cases of diarrhea the stomach empties more rapidly than normally. Alvarez¹ has pointed out that the introduction of material into the colon may retard the progress of food passing down from above, and radiologists have known that enemas must not be given immediately after a barium meal if they are to determine satisfactorily the motor activity of the stomach.

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A clinical study of the gastric emptying time in patients with colitis, who had been seen in the Roentgenological Department of the Cleveland Clinic, was made. In only fourteen of these cases had the emptying time been determined. In these fourteen cases the stomach emptied in six hours, which is the average normal time. The colitis in these cases was not severe, being cases of spastic colitis. None of these cases had a marked diarrhea.

II. EFFECT OF COLITIS ON THE GASTRIC SECRETION

The subject of gastric secretion in colitis has received considerable attention in medical literature chiefly because one of the types of "colitis" is thought to be due to gastric disturbance, the clinical condition being called "gastrogenic diarrhea." Gant,¹³ in his textbook states that "gastrogenic diarrhea" may be caused by achylia gastrica, subacidity, hyperacidity, atony, motor insufficiency, or a malignant growth. Kantor and Sagal¹⁴ studied severe forms of colitis without organic changes and state that 50 per cent of the patients complained of aggravation of symptoms when food was taken. From gastric analysis on such patients, Kantor found that an achylia or hypoacidity was usually present. Bowen and Aaron¹⁵ studied a series of diabetes cases which had uncontrollable diarrhea and found subacidity in most of them. Lunding¹⁶ studied the acidity and water content of feces in cases of "gastrogenic diarrhea" and found no appreciable variation from the normal. He gave a number of these patients hydrochloric acid by mouth, but this did not affect the acidity of the feces nor their water content.

Matsuyama¹⁷ gave sodium bicarbonate enemas in man and noticed a slight depression in gastric secretion. If the patient ate a meal after the sodium bicarbonate enema the secretion was augmented slightly, but the variations were all within physiologic limits. Ivy and Javois¹⁸ found that hydrolyzed proteins and the products of digestion stimulate gastric secretion when introduced into the intestine but that if they caused diarrhea the secretory response was reduced or abolished.

Results—The Pavlov pouch dogs were given the same meal as that used for studying the emptying time of the stomach except that the barium was omitted. The continuous secretion for one hour was obtained as a control after which the meal was given. The gastric juice was then collected at hourly intervals for seven hours. In a few instances, usually on the day after the colitis had been produced the animals would eat only part of their meals. When the dogs vomited their meals no determinations could be made.

Table I shows a normal response to a meal. During the first hour after feeding the dogs all showed free hydrochloric acid in the gastric secretion. After colitis had been produced by mustard oil, no free acid appeared in the gastric secretion until the second hour after the meal was given (Table II). The total output of the hydrochloric acid was also diminished. After the severe colitis had been produced, the dogs did not secrete any free acid until the third hour after the meal was given (Table III). In severe colitis the amount of gastric secretion was markedly diminished, as was the total output of hydrochloric acid.

TABLE I
RESPONSE OF A NORMAL DOG TO A STANDARD TEST MEAL

PROCEDURE	TIME HR	CC	FREE HCl PER CC	TOTAL HCl PER CC	TOTAL OUTPUT HCl MG PER HR
Control secretion	1	20	00	00183	0366
Standard test meal	2	40	00730	01368	5472
	3	55	02736	03646	20053
	4	80	03728	04375	35000
	5	60	03728	04375	26250
	6	40	04010	04466	17864
	7	20	03463	04193	8386
	8	20	01276	01824	3648
Total	8	335			117039

TABLE II
RESPONSE TO A STANDARD MEAL AFTER COLITIS IS PRODUCED BY MUSTARD OIL

PROCEDURE	TIME HP	CC	FREE HCl PER CC	TOTAL HCl PER CC	TOTAL OUTPUT HCl MG PER HR
Control secretion	1	10	00	00365	0365
Standard test meal	2	20	00	01276	2552
	3	30	00912	02371	7113
	4	40	02558	03646	14594
	5	50	04558	05470	27350
	6	30	03646	04375	13123
	7	30	03281	04010	12030
	8	20	02558	03463	6926
Total	8	230			85045

TABLE III
RESPONSE TO A STANDARD MEAL AFTER SEVERE COLITIS IS PRODUCED BY SILVER NITRATE
(2 PER CENT) AND FORMALIN (2 PER CENT)

PROCEDURE	TIME HR.	CC	FREE HCl PER CC	TOTAL HCl PER CC	TOTAL OUTPUT HCl MG PER HR
Control secretion	1	05	0000	0000	0000
Standard test meal	2	10	0000	00183	0183
	3	20	0000	00274	0548
	4	50	03190	04010	20050
	5	30	03281	04102	12306
	6	30	03007	03646	10938
	7	26	02736	03646	9479
	8	20	02280	03190	6380
Total	8	191			59884

TABLE IV
EMPTYING TIME OF STOMACH WITH MILD AND SEVERE COLITIS

DOG	NORMAL EMPTYING TIME, 3 TRIALS		EMPTYING TIME WITH MILD COLITIS, 3 TRIALS		EMPTYING TIME WITH SEVERE COLITIS, 3 TRIALS
	HR.	MIN	HR	MIN	
1	6		5		6 hr plate, 50% retention
2	6	10	5		6 hr plate, 60% retention
3	6		5		6 hr plate, 75% retention
4	6	15	4	45	6 hr plate, 30% retention
					dog vomited
5	6		5		6 hr plate, 50% retention

About five days after the mustard oil colitis had been produced, the dogs secreted a normal amount of gastric juice, but the maximal secretion was not attained until the seventh hour, whereas in the normal dog, the maximum secretion was attained at the end of the third hour after eating. This delayed response also occurred when severe colitis was produced with silver nitrate and formalin. In about ten days after the production of the colitis, the gastric secretion had returned to normal.

DISCUSSION

From the above data it can be seen that the colon can influence the emptying time of the stomach. The severity of the lesion present determines what this effect will be. In milder forms of irritation, the stomach empties faster than normal. This result may be compared to the observation of Carman and Moore⁹ who found that in patients with diarrhea the stomach emptied faster than normal.

When the colon was irritated severely, a delay in the emptying of the stomach was noted. In several instances the dogs vomited their meals shortly after eating. This vomiting may be attributed to pylorospasm, or decreased digestive motility of the stomach. These effects are probably due to visceral reflexes, which cause either a spasm of the pyloric sphincter or atony of the stomach.

The difference in the effect of a mild and severe colitis may be explained as follows. In cases of mild colitis, diarrhea is the chief effect, and the rate of emptying is more rapid than normal because of the diarrhea. This is supported by the clinical observation of Carman and Moore, and by observations of Fauley and Ivy¹² and those of Yesko and Mann,²⁴ who found a decreased emptying time of the stomach after ligation of the pancreatic ducts, and after starvation. Ligation of the pancreatic ducts causes an intestinal indigestion associated with frequent stools and diarrhea. The diarrhea causes a partial starvation, which is an important factor in decreasing the gastric emptying time.

In the cases of severe colitis, one must consider the effects caused by reflexes and toxemia from a markedly inflamed colon, as well as the effects of a decreased appetite. The decreased appetite and hunger are due to possible toxemia and to unpleasant sensations from the colon. The absence of appetite and hunger would act to delay the emptying of the stomach. Since it is well established that distension or irritation of the colon inhibits hunger motility through a visceral reflex mechanism, it is very likely that in cases of marked inflammation of the colon, this mechanism operates to inhibit gastric peristalsis and to cause pylorospasm. In other words, in mild colitis the diarrhea or partial starvation factor is sufficiently positive to submerge any uncomfortable sensations from the colon. But in severe colitis the toxemia, discomfort and other inhibitory mechanisms are sufficiently negative to submerge the positive effect of diarrhea and partial starvation.

CONCLUSIONS

Gastric secretion is depressed in both mild and severe colitis, because the negative factors of discomfort, diarrhea and toxemia, submerge any positive effect that partial starvation may have on the appetite secretion. In this con-

nection it is striking that the negative factors are also sufficient to submerge the positive effect of more prolonged contact of the food, with the stomach, which under normal conditions enhances gastric secretion

The results show that a mild colitis hastens slightly the emptying of the stomach, but that a severe colitis retards the emptying. After recovery from the colitis, the emptying time of the stomach returns to normal

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ISOHEMAGGLUTININS IN THE LOWER ANIMALS*

By GEORGE B. LAWSON, M.D., AND KNOWLTON T. REDFIELD, D.V.M.
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THERE has been considerable discussion during the past twenty years regarding the phenomenon of hemagglutination in the lower animals, though comparatively little has been published.

Blood transfusions were resorted to in man by Denys as early as 1657, though it was not until the present generation that our knowledge of blood groups put transfusion on a sound basis. It is needless to say there were many fatalities during the early attempts at blood transfusion. This was true to such an extent that transfusions were forbidden for a time in France. Since that time enthusiasm has alternated from low to high tide regarding this therapeutic measure. Bischoff revived the interest in this procedure in 1835 by his use of anticoagulants, though at first these were quite toxic. Landsteiner in 1900 made a most important contribution in his discovery of definite blood groups. He, however, felt that there were but three groups. Hustin and Lewisohn found a relatively nontoxic anticoagulant in the form of sodium citrate in 1914. These last two contributions at last served to put transfusion on a firm scientific basis and enlighten the world regarding the phenomenon of hemagglutination. Jansky in 1907 and Moss in 1910 added the fourth group to the above classification.

Fishbein in 1913 reported researches concerning "Iso Agglutination in Man and Lower Animals." His conclusions are as follows: "It appears that in man there is a distinct iso agglutination grouping possible, that in other mammals, iso agglutination is present but according to no special order, and that in frogs, as representing amphibians it appears to be absent." He also suggests that when facilities permit, tests be made on a wider variety of species including monkeys and higher apes, to see whether any definite gradation exists.

E. C. MacDowell and J. E. Hubbard reported an absence of iso agglutinations in mice in 1922. Ottenberg, Robdenberg and many others reported most interesting findings along similar lines at about this same time. Snyder of Massachusetts published a report of researches on "Iso Hemagglutinins in Rabbits" in 1924. He found no consistent iso agglutinations present. Walseh in 1924 reported on "The Blood Interrelationship of Horses, Asses and Mules." Also on "Hemagglutinins in Horses." This author found that the blood of horses cannot be grouped in accordance with the results of agglutination because of the autoagglutinations present.

In the human there has been considerable dispute as to when these hem

agglutinins appear. Some investigators claim that the hemagglutinins are never present at birth but may appear any time within the first two years of life. Others claim to have demonstrated them immediately after the birth of the infant. The majority, however, feel that they do not appear for the first three to six months.

Once present these groups are apparently fixed and remain so for life. Some have claimed, however, that prolonged etherization may alter these groups, though most investigators feel this to be a pseudoagglutination.

Because of the contradictory reports from various sources we have attempted the following work:

1. The determination of the presence or absence of auto- and iso-agglutination in animals of the same species. For this purpose twelve adult guinea pigs were bled by intracardiac puncture. One per cent sodium citrate was used as an anticoagulant for the cell suspension. The cells of each pig were carefully washed and the serum drawn off after centrifuging. The technique employed for all procedures with serum and cells was exactly the same as that used with the human subject.

The following is the arrangement for the macroscopic tests used in each instance, which is followed by microscopic examination as well:

4 drops guinea pig 29 serum	vs	2 drops guinea pig 34 cells
4 " " " 34 "	"	2 " " " 29 "
4 " " " 29 "	"	2 " " " 29 "
4 " " " 34 "	"	2 " " " 34 "
4 " " " 29 cells	"	2 " normal NaCl
4 " " " 34 cells	"	2 " normal NaCl
1 c c NaCl to each tube. Incubate two hours.		

In this series of 12 guinea pigs there was no evidence of either agglutination or hemolysis. Four of these pigs were adult males, eight were adult pregnant females.

The above series was repeated with rabbits (three) all adult males with the same result, no agglutination or hemolysis.

2. The presence or absence of agglutinins in heterologous species. The twelve guinea pigs were next tested against the rabbits with rather interesting results. There was no uniform agglutination or hemolysis, as one might expect when dealing with heterologous species. The serum of Rabbit 49 hemolyzed the cells of Guinea Pig 23. The serum from this pig, however, had no effect upon the cells of Rabbit 49. The serum of the same guinea pig (23) showed a two-plus agglutination (approximately 50 per cent of the cells being affected) when in contact with the cells of each of the other two rabbits. It failed to show any reaction with one rabbit. The serum and cells of Guinea Pig 41 (a pregnant animal) showed no reaction with three of the rabbits, and a two-plus reaction with three others.

The serum of Guinea Pig 84 (also a pregnant animal) showed a complete hemolysis with the cells of the three rabbits just referred to which had shown no reaction when tested against Guinea Pig 41. The serum showed both an

agglutination and hemolysis with the other three rabbits. There were no homologous agglutinins or hemolysins among the rabbits' sera and cells.

We feel from the above work that there is no homologous grouping in these animals and that there may be a specific, though not uniform, heterologous grouping. Obviously the pregnancy of the pigs and the age of the animals may have some bearing on these reactions. Both of these factors are being studied further.

The next series attempted was also with heterologous sera, human blood being used with that of guinea pigs. Here we found constant agglutination. Following this work we wished to determine the presence or absence of agglutinins and hemolysins in other species. Beginning this work we took a series of twelve monkeys testing for homologous agglutinins and hemolysins. Using Monkey 1 with each of the other eleven there was no hemolysis or agglutination shown. Using Monkey 2, this blood showed only a trace of agglutination with 7 at the end of two hours but agglutination was rather marked at the end of twenty-four hours ice box incubation. The same was true to a slightly less degree when the serum and cells of 2 and 11 were brought together. Monkey 3 with 4 and 3 with 12 showed similar reactions.

CELLS		SERUM							MONKEY			
Monkey	1	2	3	4	5	6	7	8	9	10	11	12
1		0	0	0	0	0	0	0	0	0	0	0
2			0	0	0	0	+	0	0	0	+	0
3				+	0	0	0	0	0	0	Tr	+
4			Serum Unsatisfactory									
5						0	0	0	0	0	0	Tr
6							0	0	0	0	0	0
7								0	0	0	+	+
8									0	0	0	0
9										0	Tr	0
10											0	0
11												0

SERUM		CELLS							MONKEYS			
Monkey	1	2	3	4	5	6	7	8	9	10	11	12
1		0	0	0	0	0	0	0	0	0	0	0
2			0	0	0	0	+	0	0	0	+	0
3				0	0	0	0	Tr	0	0	Tr	Tr
4			Serum Unsatisfactory									
5						0	0	Tr	0	0	Tr	0
6							0	0	0	0	0	0
7								0	0	0	Tr	Tr
8									0	0	0	0
9										0	0	0
10											0	+
11												0

Each of the same animals was next tested with each of the four human groups. In this series our reactions seemed to be most marked and it was felt that there was a possibility of groups similar to the human despite the work of Walsch, Snyder, and others to the contrary.

CELLS		SERUM (HUMAN)			
MONKEY	GROUP 1	GROUP 2	GROUP 3	GROUP 4	PROBABLE GROUP
1	+++	++	+	+++	3
			Hem +++		
2	++	++	0	+++	3
3	++++	+	++	+++	2
4	++++	0	+++	+++	2
5	+++	+	++	+++	2
6	+++	+	++	+++	2
7	+++	++	+++	+++	4
8	+++	+	+	+++	2
9	+++	+	++	+++	2
10	+++	+	++	+++	2
11	+++	+	+	+++	3
12	++	0	+	0	1

From the above series it was thought that our weak reactions were probably due to group agglutinins. Because of this suggestion of grouping in this series an effort was made to absorb nonspecific or group agglutinins with the corresponding sera. This we attempted in each case. We were successful in some cases and unsuccessful in others. We felt that our unsuccessful attempts were due to the fact that for lack of time the serum of each monkey and each human could not be titrated to determine its individual agglutinin content.

We feel that there is a possibility of a grouping of monkeys which may be comparable to those present in human serum, though this is not definite and will require further study. We feel that this is not definite since when the cells and serum of each monkey just referred to were tested against the others of a theoretically different group, we failed to get agglutination or hemolysis.

We wish to express our sincere appreciation for the help and cooperation afforded us in this work by the U S Hygienic Laboratory in Washington, D C, and to the men of the Naval Hospital from whom we obtained our blood of known grouping, and also to Miss Gortrudo Thomas who did most of the work with the guinea pigs and rabbits.

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A COMPARISON OF BLOOD PRESSURE, BLOOD UREA NITROGEN, PHENOLSULPHONEPHTHALEIN, AND URINE TESTS IN THE AGED

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THE difficulty encountered in carrying out the more complicated kidney functional tests in dispensary and out patient work led us to attempt an evaluation of the most easily determined estimations of kidney efficiency by the four simplest procedures blood pressure, blood urea nitrogen, phenol sulphonephthalein test and urine examination

Our study was confined to a group of persons who were seventy or more years of age because of the fact that the kidneys of such people are presumed to show some pathologic alteration. The most common change is arteriosclerosis, the kidneys in such condition showing some finer or coarser scarring. However, in spite of generally existent kidney abnormalities among the aged the majority of patients we observed gave no symptoms whatever of any kidney condition and with a few exceptions, were not severely ill.

Since the group with which we were dealing consisted mostly of patients who were dispensary subjects, it was not subject to the dietary control under which Heloun¹ conducted his studies, showing the effect of diet on renal function, nor are the blood urea nitrogen figures fasting estimations.

In analyzing this group of cases, we attempted to establish normals for blood pressure. We were able to find only three tables of actual estimations for the aged. The first, Table I by L. M. Bowes, which is quoted by Norris, Bazett, and MacMillan² is as follows for both men and women.

TABLE I
AFTER BOWES

Group I	70 to 74 years	Systolic 160	Diastolic 86
II	75 to 79 "	" 166	" 86
III	80 to 84 "	" 175	" 84
IV	85 to 89 "	" 170	" 90

The second table, Table II, which Wiggers³ quotes from Norris² arranges the pressures of men and women separately.

The third Table III, and following study by Wildt⁴ gives lower figures than those obtained by Bowes and Norris. These results, as Vaquez and Laidlow⁵ suggest, may possibly be due to the Riva Rocci type of instrument which Wildt employed.

Norris and Landis⁶ state that a constant systolic pressure above 160 mm

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TABLE II
AFTER NORRIS

WOMEN			
AGE IN YEARS	NUMBER EXAMINED	SYSTOLIC	DIASTOLIC
70 to 74	29	158	83
75 to 79	24	170	88
80 to 84	16	183	85
85 to 89	7	170	90
90 to 94	3	137	80
MEN			
AGE IN YEARS	NUMBER EXAMINED	SYSTOLIC	DIASTOLIC
70 to 74	10	166	91
75 to 79	14	159	89
80 to 84	11	163	84
85 to 89	0	—	—
90 to 94	4	145	81

TABLE III
AFTER WILDT

AGE IN YEARS	NUMBER EXAMINED	SYSTOLIC	DIASTOLIC
70 to 74	♂ 32	143	80
	♀ 37	150	81
75 to 79	♂ 31	139	67
	♀ 26	155	79
80 to 84	♂ 13	149	71
	♀ 26	147	84
85 to 89	♂ 6	163	83
	♀ 8	161	85
90 to 94	♀ 4	130	60

mercury, or a diastolic of 100 mm, is pathologic at any age Woley⁹ agrees with this statement as regards the systolic pressure Symonds¹⁰ says that the average for sixty years and over is 135.2 systolic and 86.9 diastolic Francis Ashley Faught¹¹ suggests a formula in which the normal average systolic blood pressure at age twenty is 120 mm, a millimeter of mercury is added for each additional two years, making a reading of 145 systolic for age seventy

We have followed Bowes' table in establishing normal blood pressures Although it may be a bit higher than the other estimations the averages are for the composite group of men and women, as is our study

The blood urea nitrogen has been considered normal if less than 20 mg per 100 cc of blood This normal is based upon Osler and MacCrae,¹² Nelson's *Loose-Leaf Living Surgery*,¹³ and Folin, as quoted by Musser and Kelly¹⁴ Blumer¹⁵ and Christian¹⁶ place the normal a bit lower, both stating that the blood urea nitrogen in a fasting individual should not exceed 15 mg

Normal figures for the two-hour elimination of phenolsulphonephthalein vary Beaumont and Dodds¹⁷ give 70 per cent as the two-hour normal Rowntree and Geraghty,¹⁸ Kolmer-Boerner,¹⁹ and Cushny²⁰ report a minimum normal of 60 per cent In the Peter Bent Brigham Hospital²¹ 50 per cent is taken as the standard for normal, while Osler and MacCrae²² state that an output of 40 per cent to 45 per cent is considered within the normal limit by many observers We arbitrarily have taken anything under 55 per cent to be abnormal for the two-hour period if the dye is given intravenously, ten minutes more being allowed if given intramuscularly

TABLE IV

CASE	AGE	B P	B U N	TOTAL PER CENT PSP 2 HR.	URINE
1	75	240/120	26	57	Ft Tr Alb Few Hyal and Lt Gran Casts
2	74	204/70	14	30	Ft Tr Alb Few Hyal Lt Gran 50 60 WBC
3	72	202/115	21	15	Ft Tr Alb Few Hyal and Lt Gran
4	72	200/100	21	40	Very Ft Tr Occ WBC
5	75	200/100	12	17	Very Ft Tr 0 Casts
6	71	185/100	21	30	0 Alb 0 Casts
7	70	185/100	12	75	0 Alb 0 Casts
8	70	185/96	21	22	Tr Alb Occ Hyal
9	75	175/100	11	70	0 Alb 0 Casts
10	78	175/80	25	25	0 Alb
11	80	170/108	36	30	Tr Alb 8 10 WBC
12	72	170/100	15	70	0 Alb 0 Casts
13	80	170/90	12	65	0 Alb 0 Casts
14	74	170/88	21	20	Ft Tr Alb Few Hyal and Lt Gran
15	77	170/80	23	35	Lt Cloud 0 Casts
16	70	170/75	21	47	0 Alb 0 Casts
17	70	165/120	16	35	0 Alb 0 Casts
18	72	165/70	19	65	Ft Tr Alb 0 Casts
19	75	160/95	16	30	Tr Alb Occ Hyal
20	76	160/70	25	70	Tr Alb 10 15 WBC
21	88	160/60	12	40	0 Alb 0 Casts
22	73	158/75	13	48	0 Alb 0 Casts
23	78	155/90	28	45	0 Alb Few Gran
24	75	155/42	105	Trace	Tr Alb 0 Casts
25	71	153/95	36	40	Lt Cloud 0 Casts
26	72	153/75	12	50	0 Alb 0 Casts
27	76	150/90	11	40	Tr Alb 0 Casts
28	75	150/70	26	55	0 Alb
29	76	145/75	21	48	0 Alb 0 Casts
30	74	142/92	36	15	0 Alb
31	74	140/80	23	16	Tr Alb Hyal
32	74	140/70	17	10	Very Ft Tr Hyal and Lt Gran

TABLE IV—CONT'D

CASE	AGE	B P	B U N	TOTAL PER CENT PSI 2 HP	URINE
33	73	138/80	18	60	0 Alb 0 Casts
34	75	138/88	16	70	Trace Alb 100 150 W B C per HPF
35	80	132/78	13	70	Tr Alb Loaded C, W B C
36	80	132/70	25	50	Tr Alb 10 20 W B C
37	76	130/70	21	28	Ft Tr Alb Few Hyal Casts
38	72	130/70	26	42	Tr Alb Loaded C, W B C
39	75	130/65	18	50	Tr Alb Few W B C
40	75	130/50	28	26	0 Alb 0 Casts
41	70	128/65	29	35	Tr Alb 0 Casts
42	70	125/60	12	55	0 Alb
43	70	125/45	19	50	0 Alb 0 Casts
44	77	124/50	17	25	Tr Alb Occ Hyal
45	71	120/95	18	65	Tr Alb Few Lt Gran
46	74	120/80	25	3	Tr Alb Occ W B C
47	72	120/60	14	45	Tr Alb, Many Hyal Few Lt Gran
48	71	115/65	22	45	0 Alb 0 Casts
49	73	110/75	14	55	0 Alb 0 Casts
50	70	108/75	10	84	0 Alb 0 Casts

While a very faint trace of albumin in persons of these ages may be of no significance, we have considered all urines abnormal which showed either the slightest albumin or casts, with the exception of those loaded with white blood cells

Table IV is a tabulation of our findings according to the systolic blood pressure readings. Where there was more than one estimation, the average was used, and the urine specimen which was most abnormal was recorded.

It will be seen that the systolic blood pressure is abnormal in only 32 per cent of the cases cited, the diastolic in 42 per cent, while the blood urea nitrogen is abnormal in 50 per cent of the cases, the urine in 54 per cent and the P S P in 70 per cent.

It is interesting that 12 per cent of the cases showed nothing abnormal, while 10 per cent of them were abnormal in all of the tests. When the other tests were normal, there were only three abnormal systolic blood pressures, the blood urea nitrogen being abnormal only once, the phenolsulphonephthalein four times, and the urine in two cases in which the other estimations were normal without exception.

SUMMARY AND CONCLUSION

A group of 50 individuals past the age of seventy years were studied in the out patient clinic to determine first if old age is often responsible for the usual manifestations of nephritis, and second, presuming such to be the case which, if any particular functional disturbance was of the greatest importance

From this study it may be assumed that in out patient work it is impossible to rely upon any single test of kidney function one may be abnormal with the others normal The test most difficult to perform in the dispensary the phenolsulphonephthalein, is the one most likely to show abnormalities the blood pressure, the least

In an individual past the age of seventy 88 per cent will give evidence of renal involvement based upon one or another of the criteria of elevation of blood pressure a lowering of phthalein output an increase in blood nitrogenous waste products and the presence of albumin and casts in the urine

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A MODIFICATION IN THE METHOD OF AUSCULTATORY PERCUSSION*

BY LEANDRO M. TOCANTINS, M.D., CLEVELAND, OHIO

IN STANDARD textbooks on physical diagnosis, when the subject of auscultatory percussion is broached, the student is instructed to place the chest piece of the stethoscope over the organ, structure, consolidation or area of fluid which is to be outlined and by percussing toward or away from the instrument, be able to detect the differences in quality of the note elicited. It occurred to me that these differences in quality of the percussion note could be better made out if the bell of the stethoscope were placed not directly over the cardiac region or the suspected area of fluid, consolidation, etc., but over a known resonant area such as the lung area in the thorax or over the tympanic regions of the abdomen.

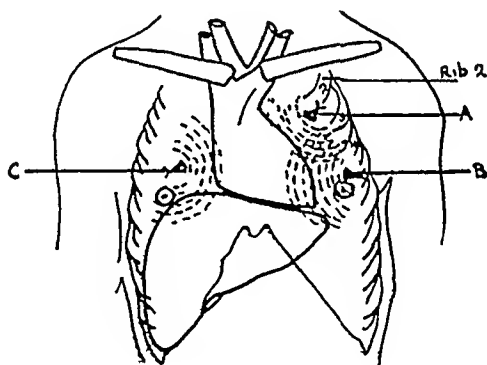


Fig 1

It is very important that, in using auscultatory percussion or, for that matter, even simple percussion, one should familiarize oneself with changes in quality rather than intensity of the note. The diminishing intensity of a note being percussed farther and farther away from the stethoscope may give rise to wrong deductions by an inexperienced observer. Not until the ear is trained to discriminate between such differences, can one afford to disregard the following precaution set by Dr. Cabot in his book on *Physical Diagnosis*:

"The line along which we percuss, when approaching an organ whose borders we desire to work out, must neither approach the chest piece of the stethoscope, nor recede from it. In other words the line along which we percuss must always describe a segment of a circle whose center is the chest piece of the stethoscope."¹

In the modification herein advocated one listens over the normal area and percusses toward the diseased or enlarged region describing segments of

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a circle as we proceed farther and farther away from the instrument. In order, for instance, to determine the slanting upper border of the heart (Fig 1), one places the bell of the stethoscope over the left second interspace (A) about one and a half to two inches inside the left nipple line and by light surface stroking or actual percussing down and away from the instrument bell, a level is reached where the note brusquely changes or entirely disappears. By sliding down the bell two interspaces and moving it a little outward to the left (Fig 1, B) one may outline the left border by the same process of light surface stroking or actual percussion. In outlining the right border of the heart the stethoscope might be placed over the right third interspace or a little above it about one inch inside the right nipple line (Fig 1 C) and percussion started toward the left until a distinct change in the note takes place. Owing to the amount of lung tissue which normally overlaps this part of the heart the definition of the right border by this method is often unsuccessful. In certain subjects it is surprisingly clear cut.

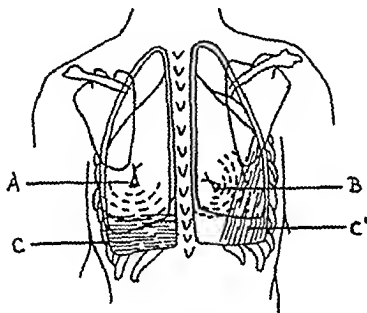


FIG 2

Without moving the bell of the stethoscope from its last position the upper border of the liver dullness may be ascertained by merely changing the direction of the stroking downward. Its lower limits may be detected by placing the chest piece a little to the right of and above the umbilicus (Fig 3 A) and stroking the abdominal surface upward along segments of a circle. Likewise the lower limits of an enlarged spleen may be arrived at if the bell of the instrument be placed a little above and to the left of the umbilicus (Fig 3 B) and the same procedure followed.

The upper limits of a pleural effusion have been (Fig 2 C) determined by holding the bell of the stethoscope over the upper resonant pulmonic area as in Fig 2 A and stroking the surface downward until the note is heard to brusquely change in quality or even disappear. To ascertain the shifting character of the dullness the patient is inclined to the right as in Fig 2 B, or to the left, and the change in C noted by using the method as outlined. The extent of a consolidation in one lung may be worked out by holding the instrument bell over an air filled section of the lung and percussing in circles

away from it. Obviously, however, one may not ascertain the boundaries of a consolidation, etc., in the left lung while holding the bell of the stethoscope over the right lung and vice versa.

Other applications of this simple process have been found in determining the height of ascitic effusions (Fig 3, *E*), of a distended bladder or enlarged uterus (Fig 3, *D*) by placing the chest piece of the instrument in the midline of the abdomen at a convenient level (Fig 3, *C*) and percussing or lightly stroking the body surface downward and away from the instrument.

In using the method one always assumes, of course, that there is continuous resonance or tympany between the area over which the instrument is resting and the border to be outlined, which is not always the case. If, however, while determining the left border of the heart no change in the quality of the note appears, one is justified in moving the bell of the stethoscope toward the axilla and renewing the attempt. The fact that the instrument was rest-

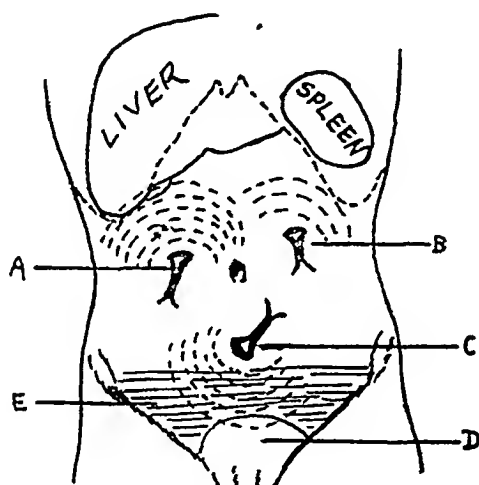


Fig 3

ing over a hypertrophied, displaced heart would explain the immutability of the note. Similar changes apply at other places as conditions so warrant.

The method must be used with full cognizance of its pitfalls in order that the results be properly interpreted. I was inclined to favor light surface stroking instead of two-finger percussion since the latter requires help from the patient or an assistant. Surface stroking yields strikingly clear and sharp results when outlining the upper limit of cardiac dullness with the chest piece of the stethoscope in the position already described.

The method is proposed for use either to corroborate previous findings or in cases where other methods have proved not altogether satisfactory, although it obviously has great defects and will sometimes, like any other method, mislead rather than help. It cannot be new as its simplicity would indicate that it must have occurred to some one before and is probably being used at present by some clinicians. A search of several books on diagnosis failed, however, to yield any reference to this method of auscultatory percus-

SUMMARY

A method of auscultatory percussion is presented differing from the one in use in that instead of placing the chest piece of the stethoscope directly over the organ, structure, area of fluid or consolidation to be outlined, the same is placed away from it over a resonant or tympanic region, and percussion made toward the area whose borders are to be defined.

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FERMENTATION OF MONOSACCHARIDS BY ORGANISMS OF THE ABORTUS MELITENSIS GROUP*

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PREVIOUS observers¹⁻⁵ have not recorded the fermentation of carbohydrates by organisms of the abortus melitensis group although McAlpine and Slanetz⁴ have reported that some strains may utilize very small amounts of dextrose, and Soule⁵ in his determination of the respiratory quotients for cultures of this group noted that 'the addition of 2 per cent glucose to the medium exerted a sparing action on the decomposition of the proteins implying a utilization of glucose.

While studying the characteristics of this group we found that acid production from certain monosaccharids might be demonstrated in a medium containing serum. Although there is still a large amount of work to be done on this problem before any definite conclusions can be drawn the results were considered to be of sufficient interest to warrant a preliminary report.

Thirty-nine strains were tested twenty-one of which were of bovine twelve of human four of porcine one of caprine and one of unknown origin. Nineteen of the bovine strains and eight of the human strains used were isolated at this laboratory. One bovine strain (215) was obtained from Dr C M Carpenter at the New York State Veterinary College Ithaca New York and one (C 339) from Michigan State College. Two of the human strains (C 343-252) were received from the Hygienic Laboratory Washington D C one (198) from the American Type Culture Collection and one (191) from Dr F S Blake Yale University New Haven Connecticut. Two of the porcine strains (203-254) were received from the Bureau of Animal Industry Washington D C one (251) from Dr Theobald Smith Rockefeller Institute for Medical Research Princeton New Jersey and the remaining one (250) from Michigan State College. The caprine strain (C 298) was received from the Hygienic Laboratory and was accompanied by the history probably isolated in Algeria originally received from Dr E Sergent Institut Pasteur d Algerie Algiers. The culture of unknown origin (C 341) labeled *B. melitensis* was obtained from Michigan State College.

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The medium used was a sugar-free 2 per cent beef-infusion agar base containing 3 per cent chlorophenol red as indicator. The final P_H was approximately 6.8. Serum (10 per cent) and filtered solutions of the carbohydrates (1 per cent) were added with sterile precautions before the agar was tubed and slanted. The monosaccharids used were arabinose, xylose, rhamnose, glucose, levulose, and galactose. The disaccharid lactose was also included.

Inoculations were made by stabbing the butt and streaking the slant. All strains which could be grown under aerobic conditions were cultured aerobically as well as in jars containing from 5 to 10 per cent CO_2 . No appreciable variation was noted in the results obtained under the different atmospheric conditions. The reactions were observed over a period of two weeks, but there was little change after one week.

The results of the tests were as follows. Arabinose was fermented by all the strains and xylose by all except one, which was of human origin. Galactose was fermented by the one caprine strain, by all but one bovine, and all but two of the human strains. Dextrose was fermented, for the most part weakly, by thirteen of the bovine strains, five of the human, and the one caprine strain. Levulose was fermented, in most instances weakly, by ten bovine and two human strains. Rhamnose and lactose were not fermented by any of the strains tested.

It is of interest to note that while twenty of the twenty-one bovine strains fermented galactose, none of the porcine strains tested fermented this carbohydrate even weakly. One of the two human strains which failed to ferment galactose (191) was isolated from a man who had handled pork products. The other (C 343) was received from the Hygienic Laboratory in Washington, D. C., as a culture of *B. melitensis*.

These observations concerning the fermentation of certain carbohydrates by members of the *Abortus melitensis* group in a medium containing serum may prove of academic interest only. However, because of the present controversial status of the differentiation of members of this group, it seems important to report any variations in their metabolic activities.

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A NEW AND SIMPLIFIED MECHANICAL AIR FILTER IN THE TREATMENT OF HAY FEVER AND POLLEN ASTHMA*

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WITH the introduction of the protein skin tests as a means of determining sensitivity, the diagnosis and treatment of allergic conditions has been placed upon a rational basis. As a result of modern treatment and from the reports in the literature, approximately 65 per cent of patients with hay fever and pollen asthma have been definitely improved or entirely relieved. It is therefore conceded that at least 35 per cent of the patients treated continue to have symptoms. It is with the latter group of patients that this communication is concerned.

The dust and miasm free chamber in which patients with asthma sleep, was introduced as a form of pure air treatment in the low regions of Holland by van Leeuwen¹ in 1924. The results of such treatment have proved successful to the extent that many of these patients were able to go about their daily work earning a livelihood in extreme comfort while others were entirely relieved of asthma. The patients who were not markedly relieved were administered nonspecific treatment with extreme benefit.

Leopold and Leopold had a specially constructed room installed in the Hospital of the University of Pennsylvania, in which it was possible to observe patients under controlled conditions of environment in relation to the presence and quantity of specifically allergic inhalation substances. Their experiments indicate that it is possible by means of their equipment to render a room dust free sufficient to keep a patient suffering with asthma from dust free of asthmatic symptoms. The cost of construction of either of the foregoing chambers is very high and therefore economically impractical for the large majority of patients.

A portable mechanical filter operated by electricity, which produced and maintained pollen free air was introduced by Cohen² for the treatment of patients with hay fever or pollen asthma. It was found that the positive pressure induced as a result of forcing the pollen free air into the room made sealing of the room unnecessary because the old air escaped through all the cracks and crevices. It was also shown that the time required by the patient to remain in the air filtered room ranged from eight to eighteen hours depending upon the individual and the pollen concentration in the atmosphere. As the season advanced a greater number of hours of freedom from exposure to pollen was required until the peak of the season after which the required number of hours gradually diminished. Patients who had received partial relief following preseasonal treatment with extracts of pollen were rendered

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free of symptoms by a residence in the filtered air chamber usually not exceeding eight hours. In these patients in whom symptoms were well established, it required from two to seven days of continuous residence in the filtered air chamber for the symptoms to subside. This apparatus has been used in the homes of a group of patients by one of us (MMP) for the past three years as an aid in the treatment of hay fever and pollen asthma. The clinical observations and the results obtained substantiate in a large measure those reported by Cohen.³ However it was found that for the average patient the relatively high cost of such a filter made its general use not only difficult but economically impossible when pollen extracts had to be administered at the same time. The replacement of the filter bag is none too easy a task.

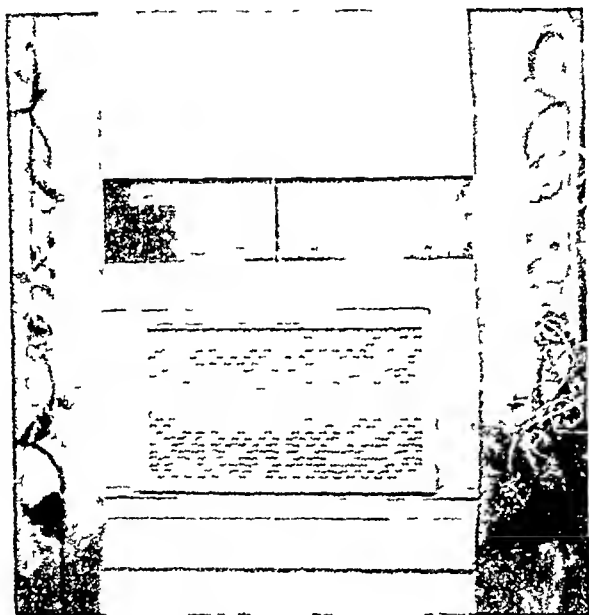


Fig. 1.—The portable mechanical air filter installed in the window. The outside view of the apparatus resembles a shutter which protects the motor and fan from inclement weather is not shown in the photograph.

Since the filter bag is expected to last an entire pollen season the efficiency of the filter after four weeks of use is definitely impaired, as evidenced by the odor and stuffiness of the room. This is enhanced in the presence of hot weather to the point of intolerance. In the hot weather experienced in Texas, Kahn⁴ found that he often had to switch over to his homemade apparatus. In spite of the improvement made in the filter bag wool fuzz is still found about the room and on the exposed vaseline-coated glass slides. The amount of space occupied and the vibration frequently encountered with this are factors of some importance when one considers the relatively small apartments occupied by many of our patients.

All of the foregoing objections in mind, an apparatus was designed for us by Mr. Charles Davies, New York City. This new and

simplified air filter, after being subjected to careful clinical⁵ and rigid laboratory tests, was found to be as clinically dependable and mechanically more efficient than any portable filter now in use

THE APPARATUS

The apparatus consists of a cabinet rectangular in shape 25 inches wide 15 inches high and 9 inches deep, which contains the filter screen, a pressure fan and an electric motor (1/30 horse power) The whole unit is so comprised



Fig 2—Shows the removal of the filter screen. A paper or linen bag can be employed in which the discarded screen is carried away.

as to fit into any sized window (Fig 1) The motor and fan which are outside the window are adequately protected against inclement weather. Electric power can be taken from any outlet in the room the current consumption being equivalent to about ten cents per day running continuously for twenty-four hours.

By means of a pressure fan air is forced through the filter screen into the room. With all the windows closed and the door slightly ajar, the air pressure created within the room is sufficient to force out pollen (when stalled

after the onset of the pollen season) and residual dust as well as prohibiting an influx of air from any source. With the motor kept working twenty-four hours a day there is a continuous stream of air free from dust and pollen.

The motor is equipped with three speeds. Low speed delivers 180 c ft of air per minute, medium speed 250 and high speed 400 c ft of air per minute. At low speed the motor is practically inaudible, medium speed produces a pleasing purr, and high speed a sound of rushing wind.

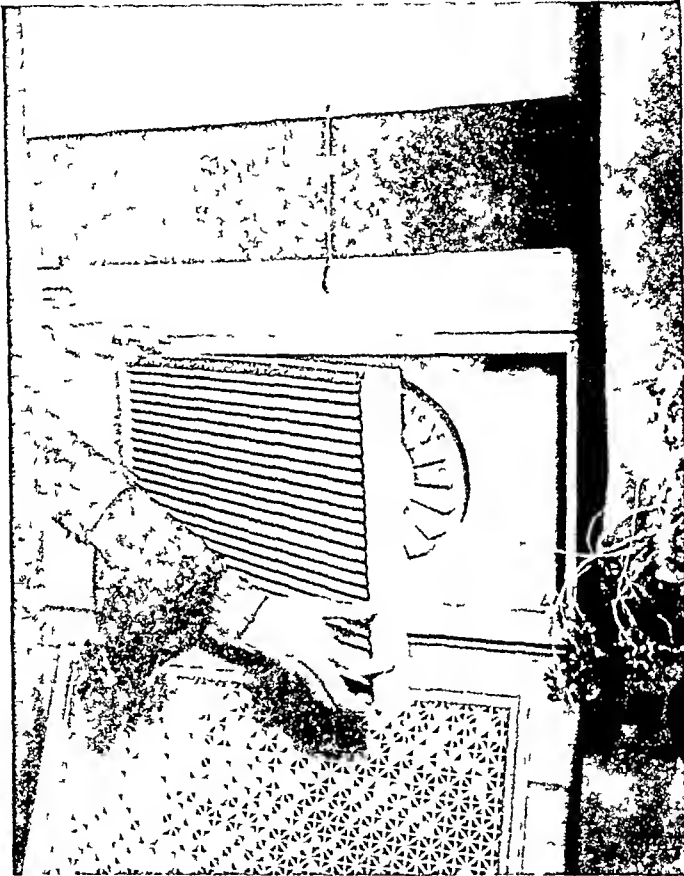


Fig 3—Shows the insertion of a new filter screen

The Ventilating Code of the American Society of Heating and Ventilating Engineers establishes as a minimum requirement 30 c ft of air per minute per person. Thus it can be seen that this apparatus delivers from three to ten times as much air as is required and that the air in a room of 2000 c ft capacity ($14' \times 14' \times 10'$) is completely changed every five to twenty minutes. This makes it possible for three to ten persons to occupy the room and at the same time have ample ventilation.

The filter screen or insert is composed of several layers of a cellulose product held between a stiff open faced netting. The screen is corrugated and has a surface area of eight square feet. The amount of dirt which floats

about in the atmosphere of the city is enormous. The accumulation of dirt menaces the efficiency of every known type of air filter by reducing the air flow. In view of this fact the apparatus is constructed in such a manner as to permit the filter insert to be easily and quickly replaced as often as may be required (Figs 2 and 3). The necessity for this became apparent during a heat wave. During such a time a large volume of air in constant motion is required for comfort. At such a time 100 c ft of air per minute was found inadequate. When the volume of air intake was raised by inserting a new filter screen the room was made comfortable.

In a test, carried out in Manhattan, with the motor running twenty four hours a day, twelve on medium and twelve on low speed and without a change of filter insert, it was found at the end of fifteen days that the air delivered into the room was 200 c ft per minute on high speed, 125 c ft on medium, and 80 c ft on low speed. Since the low and medium speeds are most frequently in use, a filter screen can be depended upon to render efficient

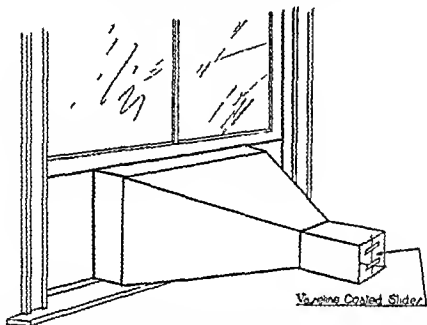


Fig 4—Shows how a concentration of all the air delivered into the room was obtained. A large funnel with an outlet measuring one half square foot was placed over the face of the filter. Vaseline coated slides were placed at an angle at the end of the outlet for twenty four hours and examined for pollen daily.

service for sixteen to twenty days. Thus three or more changes of filter screens (depending on the location and duration of the pollen season) insure continuous comfort for the patient.

This apparatus was subjected to severe tests to determine its efficiency in keeping a room free from dust and pollen. These tests were conducted in New York City and also in a suburb, Beechurst, Long Island during June, July, August, and September, 1929. The unit was installed in a room 15' x 12' x 10' (1800 c ft.). In order to obtain a concentration of all the air delivered a large funnel with an outlet measuring one half square foot, was placed over the face of the filter (Fig 4). Two ordinary atmospheric glass slides thinly coated with white petrolatum and set at an angle, were placed at the end of the outlet of the funnel for twenty four hours. Four other slides were placed face up in chosen locations about the room also for twenty four hours. Pollen counts were made simultaneously from slides directly exposed to the open air. The technique employed with reference to materials, exposure of

slides and counting of pollen was that described by Durham⁶. At no time in the period of four months were any pollen grains found on the six slides exposed in the filtered air chambers, and, at the same time, the slides remained almost entirely free from dust particles. On three separate occasions 0.17 gm of short ragweed, representing about 65,000,000 pollen grains, were thrown into the intake of the filter. A careful search of the slides placed at the exit of the funnel (Fig 4) in each instance, showed that no pollen had passed the filter screen. Short ragweed was used for these tests because it is the most important pollen clinically and at the same time one of the smallest pollens causative of hay fever and pollen asthma.

For obvious and statistical reasons the pollen count for the ragweed season of 1929 is only recorded and is shown in Chart I.

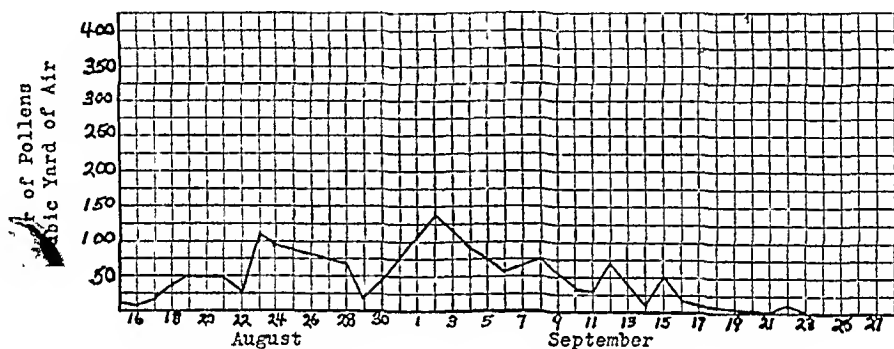


Chart I—Ragweed pollen count for 1929 of New York City and vicinity

COMMENT

The chief field of usefulness for the mechanical air filter is in the patient with hay fever or pollen asthma, who, in spite of a well-regulated course of treatment with specific pollen extracts, gets only partial or no relief from symptoms. In this type of patient the combination of both forms of treatment was found to give maximum protection thereby bringing about marked or complete relief from hay fever and asthma. We, therefore, wish to emphasize that the use of filtered air in a selected group of cases, to the exclusion of appropriate desensitization and other forms of treatment, except in an occasional instance, is not advisable.

Van Leeuwen¹ has shown that in the low regions of Holland the main causes for asthmatic and other allergic attacks were dependent upon the influence of climate, and that these factors of climate were identified with a special substance (climate-allergens or miasms), the nature of which is unknown, occurring in the air in the lower countries and lacking or present in a minor degree, in high altitudes. It is believed that among the substances which cause allergic diseases of the climate type, fungi, or rather products of fungi and yeast, are prevalent. This undoubtedly holds for many cases of asthma occurring in various sections of this country. Filtered air in these cases has proved of definite clinical and economic value.

The creation of dust-free rooms in the homes of ten patients with asthma

of the chronic refractory type by means of portable mechanical filters has been reported by Cohen.³ All these patients have remained free from asthma requiring a residence in the filtered air chamber of from twelve to fifteen hours a day.

There can be no doubt that the general use of the filtered air chamber in the diagnosis and treatment of allergic disease will do much in giving tangible relief to many patients with chronic asthma and that it will also help to create a better understanding of the subject of allergy.

CONCLUSIONS

1 The use of mechanical air filters has proved of definite value in allergic disease.

2 The use of a portable air filter as an adjunct to treatment is indicated for those cases of hay fever and pollen asthma who only obtain partial or no relief from the usual treatment with pollen extracts. The general use of a portable filter is not only restricted by its relatively high cost but is also economically impractical when specific pollen treatment is to be simultaneously administered. It should be emphasized, however, that the combined plan of treatment is the most advantageous for the patient.

3 A new and simplified portable filter which delivers dust and pollen free air is introduced. This new apparatus overcomes all the objectionable features mechanic and economic, associated with comparable air filters now in use without sacrificing efficiency of operation.

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562 WEST END AVENUE

536 WEST ONE HUNDRED AND ELEVENTH STREET

CYSTOSCOPY

For anesthesia, we have found large doses of morphine sulphate given subcutaneously so satisfactory that we have never attempted to use any other drug, and in four years' experience with dog cystoscopies we have never had to resort to a general anesthetic. As a rule, two to six grains of morphine are used, depending on the size of the dog.

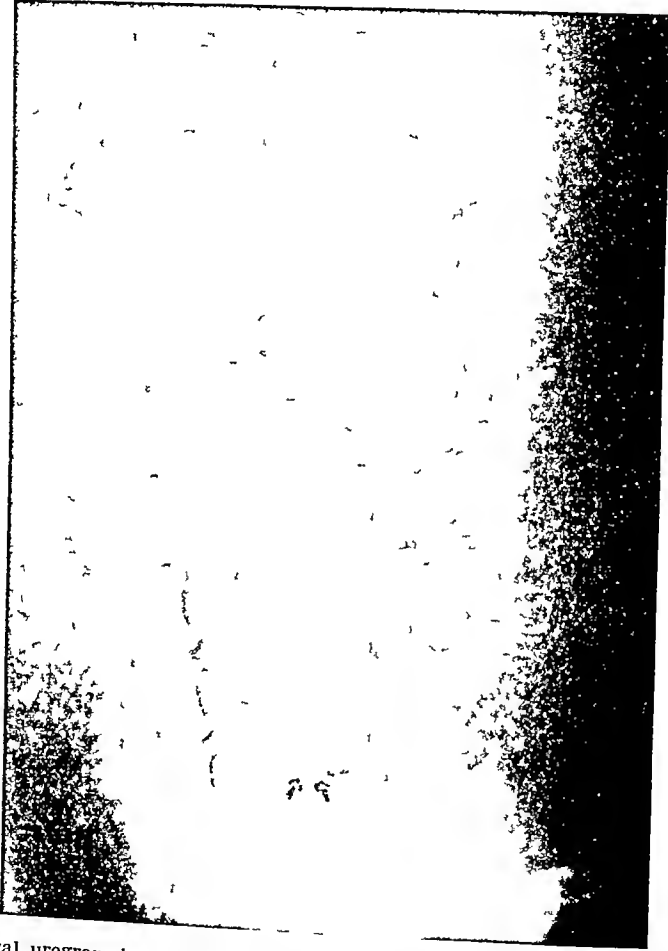


Fig 3—A lateral urogram in a living dog taken immediately after withdrawal of the ureteral catheter showing the course of the lower end of the ureter

The regular dog operating table was found very inconvenient for cystoscopy as it was difficult to keep the dog down far enough on the table. With the help of Dr C E Johnson, I designed a table as shown in Fig 6. Arms are attached to it so that the hind legs may be strapped to them and in this way prevent the dog from slipping back.

With the dog in position on the table the perineum is shaved and then painted with the "mercurochrome-acetone-alcohol" solution of Scott,* in the

*Scott J Urol 14 135 1920

is at such an angle that once more the same technical difficulty is presented. The ureter, instead of entering the bladder at an angle of from 30° to 45° with the vertical as in the human generally passes through the bladder wall at a right angle or occasionally at an obtuse angle as is seen in Fig 1 which is a drawing of a normal dog's bladder removed at operation. The course of the ureter particularly the juxta-vesical portion is diagrammatically represented in Fig 2 whereas in Figs 3 4 and 5 one can see the actual course of the ureter outlined with a medium opaque to x rays in a living dog.

TECHNIC FOR MAKING A PERMANENT EXTERNAL URETHROSTOMY

Before being able to cystoscope a male dog a preliminary urethrostomy must be made. Under ether anesthesia the dog is placed in the exaggerated lithotomy position, and his perineum and tail are shaved. A midline incision about 4 cm long is then made in the perineum at about the level of the symphysis pubis and carried downward and backward toward the anus. The

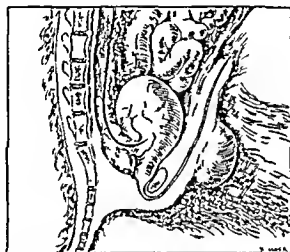


Fig 1 Drawing from an autopsy specimen with the bladder distended showing the course of the ureter as it approaches and enters the bladder.

perinealsea and bulb are divided, the bulbous urethra is located and opened longitudinally. (The location of the urethra is facilitated greatly by introducing a ureteral catheter through the urethra into the bladder.) The urethra, at the upper end of the incision, is then divided transversely, transfixed with a silk tie, and allowed to retract. The mucosa of the exposed urethra is sutured to the cut edges of the skin with interrupted sutures of fine black silk, leaving in this way a long trough of urethra with the urethrostomy opening at the lower end of the wound. The tail is so much in the way during a cystoscopic examination as well as being a constant source for contaminating the catheters that it is amputated at the second or third joint. The urethrotomies and amputation stump all heal well without infection. The sutures which do not slough out are readily removed. The opening gradually contracts down only so far we have never had enough contraction to interfere with subsequent catheterization. Ten days or two weeks after operation the wounds are well healed, and the dog is ready for cystoscopy.

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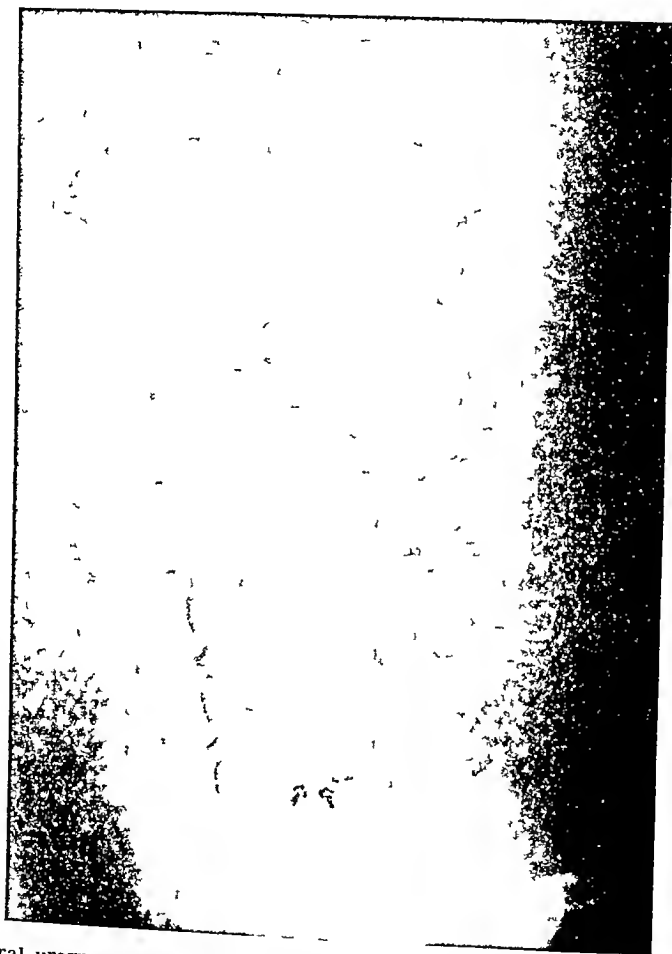


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*Scott J Urol 14 135 1925

female the vagina is swabbed out with the same solution. A small square speculum with a hole in the center is used to drape the dog that is then ready for cystoscopy.

In the male dog with an external urethrostomy, introducing a cystoscope into the bladder is a very simple procedure. In the female it is somewhat difficult, although the use of a speculum facilitates matters a great deal. The most satisfactory type of speculum is the "Collins Nasal Speculum." Intro-



Fig. 4—Another lateral urogram. Both kidneys and ureters are injected with 30 per cent sodium iodide. The course of the lower end of the ureter as it enters the bladder is shown.

ducing this into the vagina the urethral orifice is found from two to four centimeters from the outlet. Sometimes the urethra is rather small and some force must be used to pass even a No. 21 F cystoscope through it. Here one must bear in mind the fact that the urethra runs under the symphysis, parallel to the long axis of the vagina and then up into the bladder.

Our instrument of choice is a No. 21 F Brown Buerger cystoscope using two No. 5 F olive tipped, x-ray catheters. The ureteral orifices, as a rule lie fairly close together. Occasionally one seems to be above the other.

URETERAL CATHETERIZATION

With the cystoscope in the bladder sterile warm boric acid is gradually allowed to distend it until the folds of its wall are no longer visible. The more fluid there is in the bladder, the more difficult it is to locate landmarks. Withdrawing the cystoscope one soon recognizes the internal urethral orifice, the instrument is then rotated so as to look directly posteriorly. If there is



Fig 5—An anteroposterior urogram in a living dog showing the intramural course of the left ureter

only a relatively small amount of fluid in the bladder, then, turning the cystoscope about 30 degrees either to the right or left and pushing it into the bladder for about $1\frac{1}{2}$ cm, one should be looking directly at either the right or left ureteral orifice, this frequently appears as a rather delicate fold of mucosa running straight up and down, as seen in Fig 1. At times, particularly in the male dog, it appears as in Fig 7-a. In Fig 7-b the right ureteral

catheter may be seen approaching the right ureteral orifice. The angle at which the catheter approaches the orifice in this instance is the most common. From this drawing one can see that the catheterization of the ureter under these circumstances is rather difficult, and after repeated attempts to enter the ureter have failed, the ureter goes into a spasm making catheterization well nigh impossible. Sometimes the right catheter can be pushed into the ureter with the left one, and at other times it is easier to pass the left catheter up the right ureter. In Fig 7 c the catheter is seen bulging into the bladder, while about 1 cm. of it is in the ureter. In Fig 7 d the catheter has been

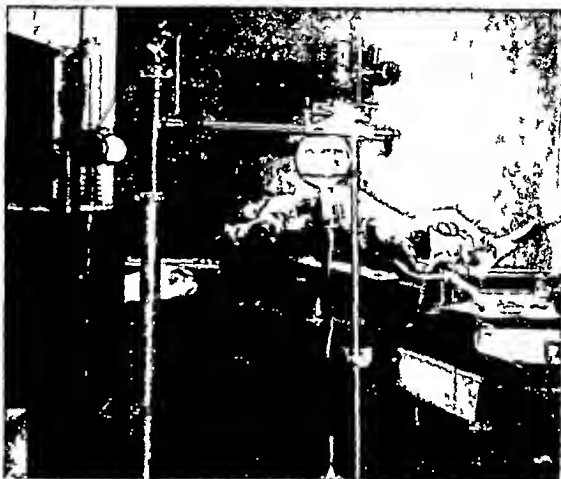


Fig 6—The dog placed on the cystoscopic table showing leg pieces, the slot for the x ray film and the construction of the table so that the dog's head can be tipped either up or down.

passed all the way to the kidney pelvis and the intramural portion of the ureter is pulled over to the side, compare this with the direction of the ureter in Fig 7 a.

The placing of the dog in the Trendelenburg position helps considerably. Because the bladder is so freely movable in the peritoneal cavity, and because the ureters enter the bladder at such an inconvenient angle, one may be unable to catheterize a ureter one day, and yet pass the instrument with perfect ease on another.

Some years ago when we first attempted cystoscopic examinations on dogs we at times spent as much as two or three hours trying to catheterize a dog's ureters without success, while a few days later—or if much damage was done, ten days or two weeks later—we were able to catheterize both ureters of the same dog all the way to the kidney pelvis in five minutes. After a few

experiences of this kind we found it far more advisable to discontinue attempts at ureteral catheterization after ten or fifteen minutes if we were not successful, as more work and less trauma was done in that way

COMMENT

Although we have no actual statistics on the matter, it is our impression that the ureters of the male dogs are more easily catheterized than those of females

Great care in technic must be used to prevent infecting the bladder or kidney pelves during cystoscopy, for this seems to occur far more readily than is generally supposed and in none of our dogs that once became infected did

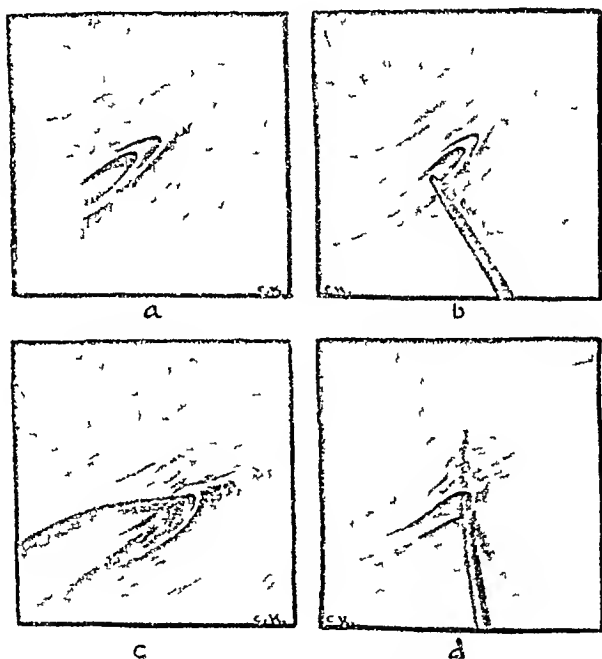


Fig. 7—Cystoscopic drawings showing successively (a) the appearance of the ureteral orifice (b) the direction in which the catheter approaches the orifice (c) the catheter entering the orifice and (d) the catheter all the way up the ureter. Note the ureteral orifice is pulled over to the side.

the infection ever clear up spontaneously. The longest, however, that we kept a dog with pyelitis and cystitis before sacrificing him was four and a half months. The infection in most of our cases has been due to the colon bacillus, occasionally to a staphylococcus.

Phthalein tests done on normal dogs, the dye being given intravenously and collected through ureteral catheters, show an appearance time of two to four minutes, and the amount of dye excreted averages perhaps just the least bit higher than in human beings, when the urine is collected for fifteen- or thirty-minute periods.

Although originally we preferred the use of an emulsion of iodized oil for our pyelograms, we have recently found this to be much less satisfactory

and more inconvenient than sodium iodide. At present we use 2 cc of a 30 per cent solution of sodium iodide in a kidney pelvis which we assume to be normal. In cases of hydronephrosis or hydroureter much more solution must be used. We have used as much as 20 cc without apparent discomfort to the dog and with no subsequent ill effects.

A NEW PUMP DESIGN FOR ARTIFICIAL RESPIRATION WITH SEVERAL VARIABLE FEATURES*

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IN WORKING with several animal preparations requiring artificial ventilation of the lungs through the period of the experiment such as the pithed cat and the heart lung preparation, a need was felt for more satisfactory methods of artificial ventilation than were available.

For oxygen consumption measurements, a closed system respiration device is necessary which will permit of normal deflation of the animal's lungs.

The Schuster (1924) pump was designed with that in view but it suffers from the important defect that it is virtually impossible to have the two barrels so adjusted that the one will remove exactly as much air as the other puts into the lungs, inasmuch as the air pressure in the lungs is not exactly the same as that in the air reservoir, usually a spirometer. Moreover if the R/Q is not unity there should be less air leaving the lungs than entering them if they are to remain at the same state of distention in inspiration or expiration at the end of a period of ventilation as they were in at the beginning. To vary the relative stroke of the two pistons with changing R/Q would be impossible as a practical method. Starling and Visseher (1927) avoided the errors inherent in a double barrel pump, one barrel for inflation and the other for deflation of the lungs by using a single barrel pump with a double mechanical valve. With this pump it was possible to inflate the lungs and allow them to deflate by their own elastic tension at a given time after inflation when a valve operated by the pump shaft was opened. This pump proved partially satisfactory but not sufficiently adjustable to be very widely useful. Its most important defect lies in the fact that one cannot vary the relative lengths of inspiration and expiration with it. A new pump has been designed and has been in actual use for three years which has obviated that defect. This pump can be put together without elaborate machine work from materials to be found in most laboratories or which at any rate can be obtained at little expense.

It consists in an arrangement for raising and lowering two mercury reservoirs 2 and 4 in Fig. 1. The reservoirs are connected by heavy rubber tubing of one half inch inside bore, to chamber 1 and the Y tube 3 respectively. Chamber 1 is fixed so that when 2 is lowered the level of mercury in 1 falls

That allows air to flow from the spirometer (not shown) through tube 9 and the water valve 6, into the chamber 1. When 2 is raised, the level of mercury in 1 rises and air is forced from the chamber. Valve 6 prevents its return to the spirometer, it is therefore forced through water valve 7 into the lungs through the Y shaped tracheal cannula 5. The air cannot escape through the trap valve 3, because, thus far during the cycle, reservoir 4 is at such a height that the mercury level in 3 obstructs any passage of air through it.

At any desired time in the cycle 4 can be made to lower so as to allow the level of mercury in 3 to fall below the level of the U, allowing the lungs to deflate themselves by virtue of their own elastic tension through the trap 3, the soda lime tower 8 and tube 9 into the spirometer, 4 is then raised by the eccen-

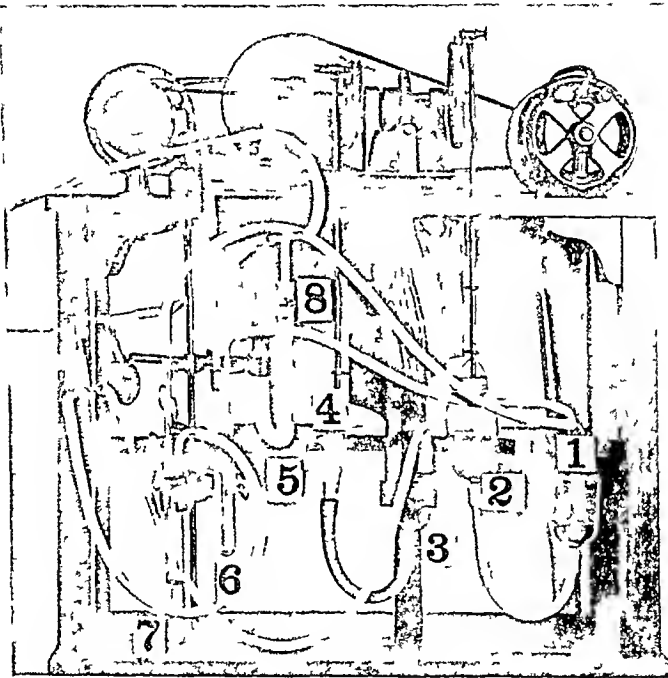


Fig 1

tric closing the trap valve 3. Meanwhile 2 is lowered by the cam, allowing 1 to be filled from the spirometer again and the whole process can be repeated.

In the pump we are using, the cams are operated from the opposite ends of the shaft of a worm drive reducing gear, driven with further pulley reduction by an electric motor of about one-twelfth h.p. The worm drive prevents any slippage which would occur with belt-driven pulleys, lifting a heavy load of mercury.

The relative points of fixation of the cams on the shaft determine the time after inspiration that expiration can occur. As pointed out above, it is very important that their relative positions can be altered. Their positions in rotation are made variable by fixing the slot holding one of them in place with a set screw on the circumference of the disc.

In order to alter the amount of air delivered per stroke by the pump it is

necessary to alter the length of the excursion of reservoir 2. This is accomplished by changing the distance of the cam from the center of the drive shaft. If it is very near the center of the disc there will be very little air delivered. Moving it in the slot and fixing it farther from the center will increase the stroke volume of the pump.

Fig. 2 shows the details of construction of the slot arrangement for fixing the cam on the wheel.

It has been found desirable to use either water valves or Tissot valves at 6 and 7 and to cover the surface of the mercury in 3 and 1 with glycerine to avoid administering too much mercury vapor to the lungs of the preparation in use.

The arrangement given in Fig. 1 is meant for a closed system for O_2 consumption measurements, but by eliminating 8 and 9, the same system has been

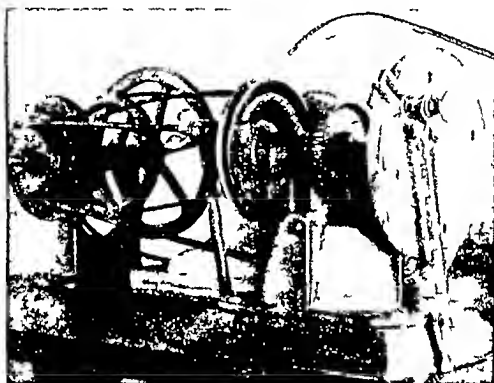


Fig. 2

found very satisfactory for artificial respiration under ordinary circumstances when there is no need of rebreathing the same air after removing CO_2 .

This pump should also be useful whenever known quantities of gases are to be administered by artificial respiration. It has shown itself to be greatly superior to the intermittent blast open tracheal cannula methods which are so difficult to regulate and with which it is always impossible to determine how much gas has actually entered the lungs, and how much has escaped through the vent.

SUMMARY

A respiratory pump is described which permits variations in the stroke volume, and in the absolute and relative lengths of inspiration and expiration, and can be adapted to oxygen consumption measurements.

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A THIONIN COUNTERSTAIN FOR LEVADITI TISSUE⁴

BY JAMES R LISA, M D, AND LADISLAW J BIRO, M D, NEW YORK, N Y

THE satisfactory demonstration of spirochetes in tissue is admittedly a difficult technical procedure attended by many pitfalls. In our hands the method of Jahnke¹ as modified for nervous tissue was found the most satisfactory. As is true of all Levaditi methods, however, it precludes study of histologic changes. In the hope of finding some way to enable one to carry out this study and still preserve the spirochetes to the greatest degree, we have experimented with many stains. The use of thionin as a counterstain was the most satisfactory and gave excellent results.

The Jahnke method is given for the sake of completeness and to obviate the necessity of referring to some other article.

JAHNKE METHOD FOR STAINING SPIROCHETES

1 Wash out in water, for one to three days, thin pieces of tissue (from 2 to 4 mm in thickness) which have been fixed in formaldehyde

2 Place in pure pyridin for one to three days

3 Wash in many changes of water until the pyridin odor almost disappears during a period of from two to three days, this is important. Then allow the tissue to remain a "few days" (emerge stage) in a 5 to 10 per cent formaldehyde solution, U S P

4 Place in water again. (The time in water this time is not stated, probably the washing should be thorough.)

5 Treat with uranium nitrate (Merck) 1 per cent solution in distilled water one half to one hour in the incubator at 37° C. The use of glass wool (lead free) under the tissue helps the penetration but is not absolutely necessary. The purpose of the uranium nitrate is to prevent the coincident staining of other elements of the nervous tissue.

6 Wash out in distilled water for one day

7 Allow to remain in 96 per cent alcohol for three to eight days

8 Wash out in distilled water until the block sinks

9 Place the tissue in a dark (amber) flask and treat it with a freshly prepared silver nitrate solution, 1.5 per cent for five to eight days in the oven at 37° C.

10 Decant the silver nitrate solution, wash the tissue slightly in water, then transfer it to a solution made up as follows:

4 per cent aqueous solution of pyrogallol	95 cc
Formaldehyde solution, U S P	5 cc

Allow the tissue to remain in the solution one or two days

11 Wash out in distilled water, embed in paraffin

The method of counterstaining follows:

1 Cut section at 3 mu

2 Remove paraffin (wash with xylol, descending alcohols, and tap water)

⁴From the Pathologic Department City Hospital Welfare Island Department of Hospitals New York N Y

Received for publication September 9 1929

¹Stevenson G S Two Recent Improvements in the Staining of Spirochetes in Nervous Tissue Arch Neurol & Psychiat 7 349-351 March 1922

- 3 Stain two minutes in thionin solution Thionin (1 1000) 1 part, distilled water 4 parts
- 4 Wash in distilled water one minute
- 5 Decolorize rapidly in two changes of acid alcohol (HCl 2 parts, 05 per cent alcohol 98 parts) until section is light yellow
- 6 Wash in two changes of distilled water, one minute each, to remove all traces of acid alcohol
- 7 Stain in thionin 1 1000, for twelve minutes
- 8 Wash in distilled water for 1 minute
- 9 Blot
- 10 Differentiate in wood alcohol acetone solution (absolute wood alcohol 3 parts, acetone 1 part) for 10 seconds
- 11 Blot
- 12 Wash quickly through aniline oil
- 13 Wash quickly through aniline oil xylol, equal parts
- 14 Clear in xylol
- 15 Mount in neutral balsam

COMMENT

Sections cut at 3 μ were found most satisfactory When thicker than 5 μ , sharp differentiation was obscured

The use of thionin in two concentrations apparently is necessary The strong solution cannot be followed by acid alcohol since the spirochetes are very frequently lost when acted upon by an acid solution for too long a time

The light yellow color obtained after decolorizing with acid alcohol is much lighter than the section freshly cut from the block If the sections are thicker than 5 μ , the end color has a brownish tinge, even this though, should be lighter than the original section

All traces of acid alcohol must be removed since any trace of acid carried over to the second thionin solution will tend to decolorize or disintegrate the spirochetes and render the stain unstable

The time element in staining with 1 1000 thionin is important Good nuclear pictures can be obtained by lengthening the time up to forty minutes The differentiation in the acetone wood alcohol mixture must then be modified The disadvantage is that too long a time in these solutions frequently causes a loss of staining or disintegration of the spirochetes

Differentiation in wood alcohol acetone is very rapid The proper end color is light yellow grey blue The substitution of ethyl for wood alcohol gives a solution which is almost as good The results after differentiating with 50 per cent ethyl alcohol are only fair

The results obtained are as follows The spirochetes are jet black, the nuclei light blue the cytoplasm is light brown yellow young connective tissue light violet, old connective tissue clear light yellow nuclei of lymphocytes plasma cells and polynuclear cells are very deep blue

ALEXIN AND ANTIALEXIC BODIES IN RELATION TO BLOOD CULTURE TECHNIC*

BY L. G. HADJOPOULOS, M.D., AND REGINALD BURBANK, M.D.,
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BUCHNER'S observation that fresh blood was naturally resistant to infection led him to postulate the presence of a protective substance in all fresh blood. The importance of this observation became evident in subsequent studies of infection and disease. Immunology as a science had its real start in the spirited study of the properties of this protective element. Our old conception of blood infections, variously designated as pyemias and sapremias gradually gave way to such definite scientific terminology as septicemias and toxemias.

The differentiation between bacteremia and toxemia is based mainly on the bacteriologic findings on culture of the blood, but, as our present methods of blood culture are as yet far from perfect the question naturally arises as to whether certain groups of so-called toxemias are not in reality true low grade septicemias. The probability of this seemed particularly strong in low grade chronic infections where the clinical evidences were also in favor of such an assumption. Our blood culture findings in subacute and chronic rheumatoid arthritis have justified us in taking this viewpoint.

In our blood culture technic we assumed that a protective substance was holding in check the active reproduction and multiplication of the specific bacteria, namely, the "alexin" of Behring. A direct evidence of this inhibition was that hanging-drop preparations of fresh blood from clinically septicemic patients sometimes showed streptococci, diplococci or bacilli even in cases where the respective blood cultures were sterile.

Subsequent investigation of "alexin" disclosed the dual nature of this substance by demonstrating both the specific immune antibody and the nonspecific complementary body. The latter fraction proved the more adaptable for experimentation and study, and our attention has in consequence been concentrated on it. This work is a study and presentation of the known anti-complementary bodies and their adaptability and value in neutralizing the bactericidal properties of the blood.

THE VARIOUS ANTICOMPLEMENTARY AGENTS

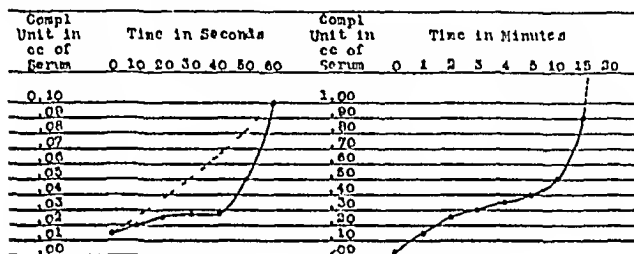
This group will be considered under the following three divisions: (1) physical agents, (2) biologic agents, (3) chemical agents.

Physical—Under this heading heat, light, and x-rays have been tested. The deleterious effect of heat on the complement was one of the first observa-

*From the Pathological Laboratories of Beth Israel Hospital, New York, and Laboratory.
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tions of students of this subject. A fifteen minute exposure of complement to 56°C is sufficient to inactivate it completely, but, as this degree of temperature for such a length of time has an equally detrimental effect on microorganisms the adoption of this method of complement inactivation is obviously contra indicated.

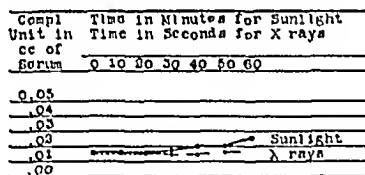
A comparative study of the rate of inactivation in terms of temperature and time was next undertaken to determine whether or not there was an optimum heat and exposure period that would not be inhibitory to bacterial growth. Table 1 shows the effect of 56°C on the complement in terms of duration of time (Graph 1).



Graph 1—The effect of heat at 56°C on the complement in terms of time

COMMENT

The broken line represents corrected figures after taking into consideration the rate of heat penetration in the particular volume of serum used. The rate of complement inactivation was rapid during the first minute of exposure to a temperature of 56°C . It then became slower up to the tenth minute beyond which time it regained its original speed until the inactivation was practically complete at the end of fifteen to twenty minutes.



Graph 2—The effect of sunlight and X-rays on complement in terms of time

The effect of light rays was studied by taking one cubic centimeter of serum spread in a flat plate which is bathed in ice water and exposed to the direct rays of the sun. Similarly another cc of the same serum was exposed to the direct radiation of X-rays. One hour of continuous exposure to the rays of the sun and one minute of exposure to the roentgen rays were ineffective in reducing complement as can be seen by Graph 2.

In the light of the above tests neither sunlight nor x-rays could be used advantageously for our purpose. The duration of the exposure effective in reducing complement was even more deleterious to the pathogenic bacteria than the effect of heat at 56° C without a corresponding destruction of complement.

With the use of heat there is, in less than a minute, a pronounced effect on the complement, a period too short to have any permanent antibacterial effect. This finding seemed hopeful, but we soon discovered that short duration exposures even at 56° C did not give a permanent reduction in complement. The gradual return of complementary value (hemolytic) after one minute's inactivation at 56° C is shown in Table I.

TABLE I

THE GRADUAL RETURN OF THE HEMOLYTIC VALUE OF THE COMPLEMENT AFTER ONE MINUTE'S INACTIVATION AT 56° C

	HUMAN SERUM IN CC							
	0.20	0.15	0.10	0.05	0.04	0.03	0.02	0.01
Original complementary titer before inactivation	++	++	++	++	++	++	++	+
Immediately after inactivation	++	+	-	-	-	-	-	-
1 minute after inactivation	++	++	+	-	-	-	-	-
2 minutes after inactivation	++	++	++	+	+	-	-	-
30 minutes after inactivation	++	++	++	++	+	+	-	-

++ Indicates complete hemolysis + partial and - no hemolysis

TABLE II

A LIST OF CHEMICALS TESTED FOR THEIR ANTICOMPLEMENTARY VALUES

	COMPLEMENT UNITS				
	4	3	2	1	0
Sodium chloride	++	++	++	++	-
Potassium chloride	++	++	++	++	-
Ammonium chloride	++	++	++	++	-
Potassium oxalate	++	++	++	+	-
Calcium chloride	-	-	-	-	-
Alcohol	++	++	++	+	-
Glycerin	++	++	++	+	-
Glucose	++	++	++	++	-
Dextrine	++	++	++	+	-
Gum acacia	++	++	++	+	-
Quinine	++	++	++	+	-
Acetanilid	++	++	++	+	-
Phenacetin	++	++	++	+	-
Hg oxychloride	++	++	++	-	-
Arsphenamine	-	-	-	-	-
Chloroform	++	++	++	-	-
Yeast	++	++	++	++	-
Pancreatin	++	++	++	++	-
Thyroxin	++	++	++	+	-
Histamine	++	++	++	++	-
Insulin	++	++	++	-	-
Peptone	-	-	-	-	-
Nutrient broth	++	++	++	++	-
Nutrient agar	++	+	-	-	-

++ Indicates complete hemolysis + partial and - no hemolysis

Biochemical—This study was limited to the anticomplementary effect of natural and artificially produced anticomplements. As natural anticomplement we used pooled inactivated anticomplementary human serum. An attempt was made to produce artificial anticomplement by injecting active human serum into guinea pigs intravenously. With both the above methods the amount of anticomplementary substance used to produce a desired effect was so great as to be prohibitive for routine work and even though such large amounts were used the results were neither permanent nor satisfactory.

Another method of inactivating complement employed was that of salting out through dialyzing membranes. The difficulties encountered in this process were mainly in keeping the cultures sterile from external contamination, and the results obtained were neither constant nor encouraging.

Chemical—The list of chemicals experimentally used comprises the salts of elements that normally exist in and are a part of the living mechanism such as sodium, potassium, ammonium calcium, iron, etc.

A group of metabolic products and certain simple nutritive substances which are eventually broken up through metabolism such as alcohol, glycerin, sugars, dextrans, peptones, etc.

A number of drugs that are most commonly used and have some bearing on the metabolic functions such as quinine, acetanilid, phenacetin, chloroform, etc.

A small list of biochemicals, the products of glandular activity. We have assembled some of this experimental data in Table II.

COMMENTS

The above ingredients were added directly to small portions of a pooled active serum in a concentration of one per cent. After subjecting the serum to the effect of the various chemicals noted above for one hour at room temperature, the hemolytic complement in the respective serum was tested and the tabulated results obtained. In repeating some of the tests and varying the concentration of the particular chemical, we observed the existence of zonal variations in indicating an optimum concentration. In the majority, however, the degree of complement inactivation had a direct bearing on the concentration. In Table II it will be noted that the outstanding anticomplementary agents are calcium chloride, arsphenamine, and peptone.

CALCIUM CHLORIDE AS AN ANTICOMPLEMENT

The anticomplementary titer of calcium chloride was determined by using varying quantities of a 10 per cent solution on a double complement unit of pooled human serum. It was found that 0.001 cc of a 10 per cent or 0.01 cc of a 1 per cent solution of calcium contained the unit anticomplementary value.

The various phases of the activity of complement are totally controlled by one cardinal serologic property, namely the relative tropism to a specific antigen antibody complex. Whether the end result is hemolysis or bacteriolysis it is simply the manifestation of cytolytic power in the particular instance. If calcium chloride can act on one of these properties (the hemolytic) it is natural to assume a similar effect on other cytolytic properties among which the

THE EFFECT OF PEPTONE ON BACTERIOTROPIC COMPLEMENT

Assuming that the anticomplementary titer of peptone is one-tenth that of calcium chloride we performed this experiment along similar lines, using a 10 per cent peptone in amounts ten times that of the calcium chloride previously employed. The results are shown in Table V

TABLE V
THE EFFECT OF PEPTONE ON BACTERICIDAL COMPLEMENT

NUMBER OF TUBES	1	2	3	4	5	6	7	8	9
COMPLEMENTARY UNITS	100	100	100	100	100		100		
ANTICOMPLEMENTARY UNITS		10	20	50	100			100	100
Human blood, defibrinated in cc	1 00	1 00	1 00	1 00	1 00		1 00		
Peptone, 10% solution in cc		0 10	0 20	0 50	1 00			1 00	1 00
Incubate 30 min at 37° C									
Inoculate typhoid, one loopful	T	T	T	T	T	T		T	
Incubate 30 min pour agar plates									
<i>Results</i>									
Growth after 5 min incubation	150	++++	++++	++++	++++	++++		- +++++	-
" " 15 "	5	++++	++++	++++	++++	++++		- +++++	-
" " 30 "	10	++++	++++	++++	++++	++++		- +++++	-
" " 60 "	5	950	450	++++	++++	++++		- +++++	-
" " 120 "	1	350	100	++++	++++	++++		- +++++	-

T a twelve-hour typhoid culture in broth The sign - indicates the absence of growth
+++ Innumerable colonies and the numerals the number of colonies per plate

THE EFFECT OF THE REACTION OF PEPTONE SOLUTION

Witte peptone solution is invariably acid A 10 per cent peptone as used in our experiments showed an acidity of about 10 per cent We reduced this acidity in a descending scale and tested the effect of the varying reactions on its anticomplementary titer The results as shown in Table VI prove that variations of reaction within the limits of our experiments were negligible in their effects on the anticomplementary titer of the peptone

TABLE VI
THE EFFECT OF REACTION ON THE ANTICOMPLEMENTARY PROPERTY OF PEPTONE

REACTION OF PEPTONE	10 PER CENT PEPTONE TITRATED IN CC				
	0 10	0 075	0 05	0 025	0 01
10 per cent acid	-	-	-	±	+
7 5 " " "	-	-	-	±	+
5 " " "	-	-	-	±	+
2 5 " " "	-	-	-	±	+
Neutral reaction	-	-	-	±	+

The sign - represents no hemolysis ± very faint hemolysis and + partial hemolysis

Irrespective of the reaction the anticomplementary titer of the 10 per cent peptone solution was between 0 01 and 0 02 cc

COMMENT

In the first column of Table VI we see the bactericidal effect of fresh human serum on typhoid bacilli The checking of growth is almost complete at the end of two hours, and there is no visible sign of multiplication following this period The second and third columns demonstrate satisfactorily the par-

TABLE VII
A COMPARATIVE TABLE SHOWING THE SUPERIORITY OF PEPTONE-AGAR
ORDINARY BLOOD CULTURES

SERIAL NUMBER	NUTRIENT AGAR	NUTRIENT BROTH	PEPTONE INACTIVATED	TYPE OF ISOLATED MICROORGANISM	SERIAL NO OF REPEAT CASES	RESULT
8	-	-	+	Strep viridans		100%
9	-	-	+	Strep viridans		100%
10	+	-	+	Typhoid bacillus		100%
11	-	-	-			100%
12	-	-	+	Diphtheroid bacillus		100%
13	-	-	-			100%
14	+	-	-	Diphtheroid bacillus	9	100%
15	-	-	-		10	100%
16	-	-	-		11	100%
17	-	-	+	Strep viridans	9, 10	100%
18	+	-	+	Strep viridans	11	100%
19	-	-	-		12	100%
20	+	+	+	Staph aureus		100%
21	+	+	+	Strep viridans	14, 15	100%
22	-	-	+	Strep viridans		100%
23	+	-	+	Pneumococcus		100%
24	+	-	-	Pneumococcus		100%
25	-	-	-		9, 10, 11	100%
26	-	-	-			100%
27	-	-	+	Strep hemolytic		100%
28	+	-	+	Staph aureus		100%
29	-	-	+	Strep hemolytic		100%

Corrected Peptone

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use Just
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solution *
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at the end of
on All three
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tial neutralization of this bactericidal property by insufficient concentrations of peptone. Complete neutralization of the germicidal value occurred in Tubes 4 and 5. The unit antibactericidal titer evidently was represented in an amount between 0.50 and 1 cc of 10 per cent peptone. A 10 per cent peptone solution with a reaction of 10 per cent acidity proved to be a satisfactory culture medium for the growth and development of typhoid bacilli as is illustrated in control Tube 8.

The application of peptone inactivation of complement for blood culture work was employed by us, and a description of the provisional technique follows.

A 10 per cent peptone is prepared, boiled, filtered, and divided into amounts of 2.5 cc each in 5 cc test tubes. After two consecutive sterilizations they are stored in the ice box ready for use. At the time of inoculation with blood they are heated to body temperature and inoculated with 2 cc of the patient's blood. This is mixed well, and after one hour of incubation, half of the mixture is transferred to 10 cc of nutrient agar, and a plate is poured. The pour plate and what remains of the peptone blood mixture are left in the incubator and examined daily for growth.

A series of such blood cultures from the hospital service properly controlled with ordinary blood culture technique is given in Table VII.

Because of the high acidity of the peptone solution, in the latter half of Table VII, the reaction will be found corrected to almost neutral. This was done in order to find out whether or not a corrected reaction would result in a higher percentage of positive findings. The results as shown in the table did not show much variation. Bacilli of the paracolon group, such as typhoid, usually require acid media and consequently did not grow well in the neutralized peptone mixture.

An analysis of Table VII shows the superiority of the peptone neutralized complement method in detecting streptococci in the blood stream. The comparison of the findings by both methods is demonstrated in Table VIII.

TABLE VIII

COMPARATIVE RESULTS OF NUTRIENT AGAR AND PEPTONE NEUTRALIZED BLOOD CULTURE WITH AN ANALYSIS OF THE TYPE OF ORGANISM ISOLATED BY EITHER METHOD

RESULTS OF FINDINGS	NUMBER OF CULTURES	PER CENT	ANALYSIS OF DIFFERENCES	AGAR POSITIVE PEPTONE NEGATIVE	AGAR NEGATIVE PEPTONE POSITIVE	BOTH CULTURES POSITIVE
Both tests positive	18	36	Str Vir & Hem		7	4
Both tests negative	17	34	Pneumococcus	1		1
Total agreement		70	Diphtheroids	1	1	1
Nutrient agar positive			Typhoid Bac	2		3
Peptone neutralized negative	5	10	Staphylococcus	1	2	9
Nutrient agar negative				5	10	18
Peptone neutralized positive	10	20	Total positives			33
Total disagreement		30	Total negatives			17
Difference favoring peptone		10	Total number of cultures			50

THE SPECIAL BLOOD CULTURE TECHNIC FOR THE DETECTION OF STREPTOCOCCI

Because of the selective anticomplementary property of peptone we have devised the following blood culture technique for the isolation of streptococci in low grade chronic infections, especially arthritis.

A 10 per cent solution of peptone (Digestive Ferments) is prepared, filtered, sterilized, and divided in amounts of 10 cc in 20 cc test tubes. After a second sterilization the tubes are stored in the ice box for future use. Just before inoculation with the blood to be cultured, the tube is heated to body temperature and neutralized with 1 cc of 1 per cent sodium carbonate solution.* To each tube of the neutralized peptone we add 2 cc of the patient's blood. This is well mixed, incubated for one hour, and the first blood plate is poured, using 2 cc of this mixture. Similarly another plate is poured at the end of twenty-four hours and also one after forty-eight hours' incubation. All three plates are left in the incubator and examined daily for the appearance of growth.

Any suspicious colony is smeared on the surface of the blood plate on which it is found, and a slide is also made and stained. After examination of the stained specimen all colonies suspected of being streptococci are planted in broth. The following morning the surface growth on the blood plate discloses the hemolytic properties of the organism while the broth culture is transplanted to differential sugar media for final classification as well as studied for biologic properties.

By the application of this technic, we have been able to isolate the streptococcus from the blood stream in approximately 10 per cent of our arthritic cases. Both hemolytic and viridans streptococci have been found and occasionally a nonhemolytic (nonviridans) growth was encountered.

6 EAST SEVENTY EIGHTH STREET

In our attempts at isolation of streptococci from stool cultures our laboratory technicians William Striefler and Katherine Kaufman have noted that while 1 per cent sodium carbonate did not manifest any deleterious effect on streptococci this concentration was sufficient to check completely the growth of gram negative flora for a period of approximately forty-eight hours. As a consequence of this observation the mixing of a small portion of stool the size of a pea in 10 cc of 1 per cent sodium carbonate solution and leaving overnight in the incubator renders the isolation of streptococci from subsequent blood agar pour plates extremely simple and easy.

PERMANENT COLOR STANDARDS FOR BLOOD BILIRUBIN*

BY M. STARR NICHOLS, PH D, AND J. WARREN JACKSON, M D, MADISON, WIS

THE discovery of the diazo reaction for bilirubin by Ehrlich (1883), and the application of this test by Hymans van den Bergh (1913, 1921) to clinical practice has stimulated work in the differentiation of types of jaundice and of certain blood diseases in which there is blood destruction and liberation of hematin. The complexity of the original method has retarded the use of this valuable test. Furthermore, van den Bergh's original method using the mercuric rhodanate standard introduces two or three definite errors, i.e., incomplete extraction of the color, concentration of the standard by evaporation of the ether, and lack of identical shade of color of this rhodanate standard with the color produced in the reaction. McNee (1925) has reported that van den Bergh now advises the use of a cobalt sulphate solution in the proportion of 2.161 gm of the anhydrous salt to 100 cc of distilled water, to replace the rhodanate standard and to equal the color of 1 unit (1-200,000) of bilirubin. While this standard compares quite well in color intensity with the rhodanate standard it is not the same shade of color as the diazo-bilirubin produced in the serum, and is difficult to use on that account. Rhamy and Adams (1928) have proposed a potassium permanganate solution but this solution in its diluted form is very unstable and cannot be employed in ampoule color standards.

In our standards we use a mixture of cobalt chloride, hydrochloric acid, and water. The hydrochloric acid changes the yellowish pink natural cobalt color to that of violet pink and produces an exact match in shade for the color produced in the quantitative van den Bergh test.

PREPARATION OF STANDARDS

A solution of pure bilirubin was prepared of the strength of 1 part of the pigment to 200,000 parts of the solution and therefore contained 1 unit (van den Bergh) to each cc of the solution. The cobalt chloride, hydrochloric acid water solution was then adjusted to provide an exact match for this color when 1 cc of this solution was treated according to van den Bergh's procedure. This mixture of cobalt thus standardized provided the standard for 1 unit of bilirubin (van den Bergh). Standards ranging from 0.5 to 10 units were then prepared in a like manner. The details of the preparation of these bilirubin solutions are given as follows. A chloroformic solution of bilirubin was made in concentration of 1 part of the pigment to 5000 parts of chloroform. From this stock solution of bilirubin the one unit strength was prepared by diluting 0.5 cc of this stock solution to 20 cc with a mixture of chloroform and alcohol. Table I shows the exact quantities used in preparing the bilirubin solutions of exact unit strength from 0.5 unit to 10 units.

*From the State Laboratory of Hygiene and the Department of Clinical Pathology, Wisconsin General Hospital, University of Wisconsin.
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Our diazo bilirubin colors were made from these bilirubin solutions in exactly the same manner as though they were serums containing these definite units of bilirubin and was as follows To 1 cc of the bilirubin solution of desired unit strength 3.25 cc of 95 per cent alcohol and 0.75 cc of freshly prepared diazo reagent were added Five minutes time was allowed to elapse and the appropriate permanent color standard was made to match the diazo bilirubin color as developed at the end of the above time interval

TABLE I
SHOWING PREPARATION OF BILIRUBIN SOLUTIONS

AMOUNT STOCK SOLUTION BILIRUBIN PRESENT IN 20 CC OF FINAL DILUTIONS	AMOUNT OF CHLOROFORM ADDED TO MAKE SOLUTION 25 PER CENT CHLOROFORM	AMOUNT OF 95 PER CENT ALCOHOL ADDED	TOTAL VOLUME OF THE BILIRUBIN SOLUTIONS OF KNOWN STRENGTH	STRENGTH OF THESE BILIRUBIN SOLUTIONS	ACTUAL AMOUNT OF BILIRUBIN PRESENT IN 1 CC OF THESE SOLUTIONS
CC	CC	CC	CC	UNITS	MG
0.25	4.75	15	20	0.5	0.0025
0.5	4.5	15	20	1.0	0.005
1.0	4.0	15	20	2.0	0.01
1.5	3.5	15	20	3.0	0.015
2.0	3.0	15	20	4.0	0.02
2.5	2.5	15	20	5.0	0.025
3.0	2.0	15	20	6.0	0.03
3.5	1.5	15	20	7.0	0.035
4.0	1.0	15	20	8.0	0.04
4.5	0.5	15	20	9.0	0.045
5.0	0	15	20	10.0	0.05

In the preparation of these standards it was found that 41 cc of concentrated hydrochloric acid to 100 cc of final solution furnished the correct acidity, and that the amount of cobalt needed was directly proportional to the bilirubin content From the above standardizations and these findings we have found it possible to duplicate the standards simply by referring to the dilution table Solutions required A 20 per cent aqueous solution of freshly crystallized cobaltous chloride ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$), concentrated by hydrochloric acid (sp gr 1.2), and distilled water Table II gives the amounts of these solutions necessary to duplicate these permanent color standards

TABLE II
FOR DUPLICATION OF PERMANENT COLOR STANDARDS

VAN DEN BERGH UNITS REPRESENTED	AMOUNT OF 20 PER CENT COBALT SOLUTION	AMOUNT OF CONCENTRATED HYDROCHLORIC ACID (SP GR 1.2)	DISTILLED WATER SUFFICIENT QUANTITY TO MAKE
	CC.	CC	CC
0	0	0	100
0.5	0.65	41	100
1.0	1.3	41	100
2.0	2.6	41	100
3.0	3.9	41	100
4.0	5.2	41	100
5.0	6.5	41	100
6.0	7.8	41	100
7.0	9.1	41	100
8.0	10.4	41	100
9.0	11.7	41	100
10.0	13.0	41	100

Use of Permanent Color Standards It will be noted that we use 1 c c of the bilirubin solutions representing units but this is diluted to 5 c c when the diazo-bilirubin color is made. Our standards are made to compare with the diazo-bilirubin colors therefore no factor is used for obtaining the end-result. The most convenient method for making the determination is as follows: Prepare the color standards according to Table II. Procure small flat-bottomed glass ampoules holding about 2 c c. These ampoules should be of clear glass, of the same diameter and provided with a long drawn-out neck for sealing. Fill these ampoules with the prepared permanent colors, label with unit contents and seal in flame. The standards so prepared keep indefinitely if moderate care as to heat and light is observed. A similar empty ampoule without neck is used for the diazo-bilirubin color in the quantitative estimation. The serum is obtained free from hemolyzed blood in the usual manner. To 1 c c of this serum add 2 c c of 95 per cent alcohol, mix well in centrifuge tube and centrifuge to separate the precipitated protein from the clear fluid. To 1 c c of this fluid contained in empty ampoule, add 0.5 c c of 95 per cent alcohol and 0.25 c c of freshly prepared diazo reagent. Wait five minutes and com-

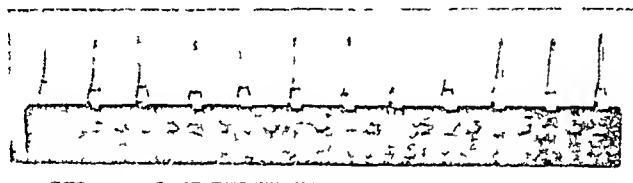


Fig 1—Illustration of permanent color standards and comparator box.

pare in comparator box (see Fig 1) with the permanent standards making your observation toward a source of diffuse light (a strip of ground glass, such as photographers use, or even a thin sheet of white paper placed back of the standards and sample will aid in the comparison). Read the units

TABLE III
BLOOD BILIRUBIN NORMAL

CASE NUMBER	ICTERUS INDEX, MURPHY'S TECHNIC (1926)	VAN DEN BERGH QUALITATIVE	VAN DEN BERGH QUANTITATIVE IRON STANDARDS UNITS	VAN DEN BERGH QUANTITATIVE PERMANENT STANDARDS UNITS	FINAL CLINICAL DIAGNOSIS AT TIME OF DISCHARGE FROM HOSPITAL
4	5	del dir reaction	10	10	Chronic endocervicitis
11	6	"	10	10	Duodenal ulcer, spastic colitis
15	5	"	10	10	Chronic appendicitis, chronic tonsillitis
16	5	"	10	10	Spastic colitis
29	5	"	05	05	Dermatitis herpetiformis
45	5	"	10	10	Parkinson's disease
58	5	"	05	05	Threatened abortion
61	5	"	05	05	Perinephritic abscess
66	5	"	10	10	Osteochondromatosis
71	5	"	05	05	Ptoisis of right kidney
					Observation

TABLE IV
BLOOD BILIRUBIN PATHOLOGIC

CASE NUMBER	ICTERUS INDEX, MURPHY'S TECHNIC	VAN DEN BERGH QUALITAT	VAN DEN BERGH QUANTITAT IRON STANDARDS UNITS	VAN DEN BERGH QUANTITAT PERMANENT STANDARDS UNITS	FINAL CLINICAL DIAGNOSIS AT TIME OF DISCHARGE FROM HOSPITAL
1	35	del dir reaction	80	80	Pernicious anemia
2	8	del dir reaction	20	20	Arteriosclerosis with hypertension
5	100	imm dir reaction	450	450	Carcinoma of pancreas
6	3	del dir reaction	04	05	Abortion.
7	8	del dir reaction	16	15	Cholecystitis
8	12	del dir reaction	25	25	Pernicious anemia
9	50	imm dir reaction	85	90	Cholelithiasis
10	40	imm dir reaction	55	55	Cholelithiasis
12	25	imm dir reaction	625	65	Acute cholecystitis
13	5	del dir reaction	08	10	Spastic colitis chronic cholecystitis
14	25	imm dir reaction	70	70	Cholelithiasis
17	10	del dir reaction	20	20	Chronic cholecystitis
19	15	imm dir reaction	30	25	Subacute cholecystitis
20	8	del dir reaction	10	10	Pernicious anemia
22	1	no reaction	0	0	Carcinoma of hepatic flexure of colon
25	15	del dir reaction	25	25	Stone in common duct
26	4	del dir reaction	10	10	Retroperitoneal abscess
27	6	del dir reaction	10	10	Chronic cholecystitis
28	5	del dir reaction	08	10	Chronic cholecystitis ovarian cyst
30	30	del dir reaction	50	50	Cholelithiasis
31	10	del dir reaction	25	25	Cholecystitis?
32	20	imm dir reaction	50	50	Chronic colitis
33	15	del dir reaction	30	30	Chronic colitis cholelithiasis
34	35	biphasic del react	100	100	Pernicious anemia
35	10	del dir reaction	20	20	Empyema strep hemolyticus B type
36	8	del dir reaction	15	15	Pernicious anemia syphilis
37	8	del dir reaction	15	15	Tuberculous meningitis
38	8	del dir reaction	20	20	Pernicious anemia syphilis
39	6	del dir reaction	10	10	Chronic cholecystitis
		del dir reaction	10	10	Pernicious anemia

TABLE IV—CONT'D

CASE NUMBER	ICTERUS INDEX MURPHY'S TECHNIC	VAN DEN BERGH QUALITAT	VAN DEN BERGH QUANTITAT IRON STANDARDS UNITS	VAN DEN BERGH QUANTITAT PERMANENT STANDARDS UNITS	FINAL CLINICAL DIAGNOSIS AT TIME OF DISCHARGE FROM HOSPITAL
40	2	del dir reaction	0.5	0.5	Carcinoma of bladder
41	6	del dir reaction	1.5	1.5	Secondary anemia from bleeding hemorrhoids, tonsillitis
42	6	del dir reaction	1.0	1.0	Pernicious anemia
43	8	del dir reaction	1.0	1.0	Chronic cholecystitis
44	1	no reaction	0.2	0.2	Carcinoma of stomach
46	6	del dir reaction	1.0	1.0	Acute cholecystitis
47	100	imm dir reaction	32.0	32.0	Acute cholecystitis
48	8	del dir reaction	1.0	1.0	Spastic colitis
50	6	del dir reaction	1.5	1.8	Arrest pul T B Pos sible chron cholecysti tis
51	6	del dir reaction	1.0	1.2	Chron spastic colitis, chronic cholecystitis
52	15	del dir reaction	2.0	2.0	Carcinoma of stomach with metastasis to liver
54	25	imm dir reaction	6.0	6.25	Syphilitic aortitis with decompensation— general anasarca
55	8	del dir reaction	1.0	1.0	Ruptured ectopic preg nancy
56	8	del dir reaction	2.0	2.0	Pernicious anemia
57	8	del dir reaction	2.0	2.0	Pernicious anemia
59	20	del dir reaction	5.5	6.0	Secondary carcinoma of liver
60	20	del dir reaction	5.5	6.0	Secondary carcinoma of liver
62	8	del dir reaction	1.0	1.0	Secondary anemia due to bleeding hemorrhoids
63	2	no reaction	0	0	Chronic colitis, chronic hypertrophic arthritis
64	2	no reaction	0	0	Nephrolithiasis
65	20	imm dir reaction	5.0	5.0	Acute cholangitis
67	6	del dir reaction	1.5	1.5	Amebiasis, histolytica
68	15	imm dir reaction	2.0	3.0	Acute cholangitis
69	4	del dir reaction	0.5	0.5	Prob chronic cholecystitis
72	4	del dir reaction	0.6	0.5	Spinal cord tumor
74	20	del dir reaction	4.0	4.0	Chronic cholecystitis
75	10	del dir reaction	3.0	3.0	Pernicious anemia.

TABLE IV—CONT'D

CASE NUMBER	ICTERUS INDEX. MURPHY'S TECHNIC	VAN DEN BERGH QUALITAT	VAN DEN BERGH QUANTITAT IRON STANDARDS UNITS	VAN DEN BERGH QUANTITAT PERMANENT STANDARDS UNITS	FINAL CLINICAL DIAGNOSIS AT TIME OF DISCHARGE FROM HOSPITAL
77	20	imm dir reaction	50	50	Chronic cholecystitis
78	50	imm dir reaction	100	100	Carcinoma of pancreas with metastasis to liver
79	50	imm dir reaction	200	200	Catarrhal jaundice
80	20	del dir reaction	30	30	Patient No 79 three days later
81	25	imm dir reaction	90	90	Chronic cholecystitis

directly from the color standards after matching. No calculation is necessary as this was taken into consideration in the preparation of the table.

Time Factor. Diazo bilirubin color increases in intensity for about the first hour but most of this color develops within the first five minutes. Our standards were matched at the end of five minutes and therefore give the most accurate results when the color is compared at that time.

CLINICAL RESULTS

Blood bilirubin was determined both by the use of these permanent color standards and by the original van den Bergh method using the ethereal solution of iron rhodanate. These comparative results are given in Tables III and IV.

SUMMARY

A simplified technic for the colorimetric determination of blood bilirubin is given. While van den Bergh's method for this determination is followed up to the colorimetric comparison this latter comparison is simplified so that a determination of blood bilirubin can be made as easily as a phenolsulphonephthalein (PSP) function test. Clinical results are given to show the degree of accuracy to be expected as compared with the old colorimeter method.

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THE HEATING OF SERUM IN THE KAHN REACTION*

By M B KURTZ, D V M, M S, LANSING, MICH

IN THE Kahn reaction, and in many of the Wassermann technics, serum is usually heated for thirty minutes at 56°C before being tested¹ The term "inactivation" is ordinarily applied to the process, although the function of the heat treatment in the Kahn reaction is apparently not to destroy native complement It is questionable whether complement plays a rôle in this reaction It is more likely, as recently observed by Nishio² in Kahn's laboratory, that the function of heating is to reduce the protective properties of the serum albumins to precipitation

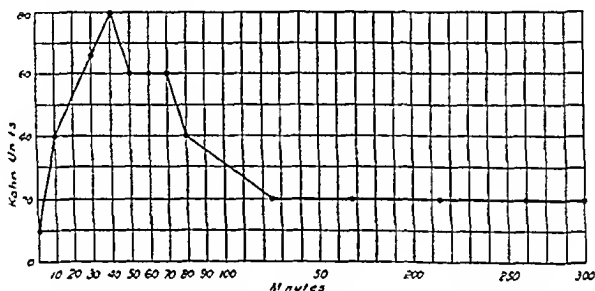


Fig 1 —Potency of serum heated at 56°C for different time periods

During the development of the Kahn test, experiments were carried out to determine the optimum heating period for serum at 56°C It was found that the sensitiveness of the precipitation reaction increased with the duration of the heating treatment, optimum results being obtained after heating for about thirty to sixty minutes at 56°C ³ Further heating at this temperature caused practically no change in sensitiveness until the heating period exceeded one and one-half hours after which a slight decrease in sensitiveness was observed A more pronounced fall in sensitiveness was induced by subjecting sera, that had already been heated at 56°C for one-half hour, to prolonged heating at 62°C Based on these experiments, the heating period chosen for the Kahn test was thirty minutes at 56°C It has seemed reasonable to suppose, however, that the satisfactory results obtained with this standard heating period might be duplicated by using a somewhat higher temperature for a period less than thirty minutes, thereby shortening the time required to carry out the test This article presents a brief summary of the experiments carried out together with the results obtained

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EXPERIMENTAL

In these studies, the Kahn qualitative and quantitative procedures were used. In the former procedure, a proportion of serum to antigen suspension was employed, corresponding to that of the third tube of the regular 3 tube Kahn test. One half the regular amounts of suspension and serum were used, namely, 0.006 cc suspension and 0.07 cc serum, because of the limited amount of individual serum available. Standard Kahn antigen was used throughout these studies. Each serum was divided into two portions, one portion being heated for thirty minutes at 56° C and serving as a control, while the other portion was heated at a higher temperature for varying periods of time. The sera were kept stoppered during the heating period in order to prevent evaporation.

Experiment 1—One portion each of 950 sera was heated at 56° C for thirty minutes, while the other portion in each case was heated for ten minutes at 60° C. The qualitative 1 tube procedure was employed in making the tests and the results recorded on a ++++ basis as is illustrated in Table I. Identical results were obtained with the two portions in 915 (96.3 per cent) of the sera used. A variation of more than + was shown by only 11 sera of which 6 showed stronger reaction when heated for thirty minutes at 56° C, and 5, on the other hand, gave stronger reactions when heated at 60° C for ten minutes.

TABLE I

EFFECT OF DIFFERENT HEATING PERIODS OF SERUM ON SENSITIVENESS OF KAHN REACTIONS

NO OF SERA	REACTION AFTER HEATING FOR THIRTY MINUTES	REACTION AFTER HEATING FOR TEN MINUTES
	AT 56° C	AT 60° C
144	++++	++++
3	++++	+++
1	++++	+
1	+++	++++
11	+++	+++
4	+++	++
3	+++	±
3	++	++++
2	++	+++
5	++	++
2	++	+
2	++	±
1	+	++
2	+	+
1	+	±
7	±	±
6	±	-
2	-	++
4	-	±
746	-	-

It appears from this experiment that with serum tested by means of the qualitative 1 tube procedure, a heating period of ten minutes at 60° C is equivalent to the regular heating period of thirty minutes at 56° C.

Experiment 2—The quantitative procedure was employed with 65 strongly positive sera, again using the heating period of ten minutes at 60° C and the

control period of thirty minutes at 56° C The results obtained are listed in Table II There was agreement in 59 (91 per cent) of the sera In the case of the other 6 sera (9 per cent) the variations did not exceed the difference between the number of Kahn units given by one serum dilution, and the number that would be given by the next higher or next lower dilutions, the sensitiveness being sometimes slightly below that of the control This experiment thus confirms the result of the previous experiment

TABLE II
EFFECT OF DIFFERENT HEATING PERIODS OF SERUM ON SENSITIVENESS OF
QUANTITATIVE KAHN REACTIONS

NO OF SERA	NO OF KAHN UNITS AFTER HEATING SERUM FOR THIRTY MINUTES AT 56° C	NO OF KAHN UNITS AFTER HEATING SERUM FOR TEN MINUTES AT 60° C
1	280	280
2	240	240
1	200	200
1	160	160
3	120	120
1	*120	80
1	*80	40
8	40	40
3	*20	40
1	*20	4
13	20	20
30	4	4

*Variations

Experiment 3—The effect of heating serum at 62° C for five minutes was then studied, using the 1-tube qualitative test with 260 sera, and the same system of controls as in the previous experiments Results identical with those of the controls were obtained in 245 (94.2 per cent) of the sera, as is shown in Table III The remaining 15 sera gave variations ranging from + to + + +, 12

TABLE III
EFFECT OF DIFFERENT HEATING PERIODS OF SERUM ON SENSITIVENESS OF
KAHN REACTIONS

NO OF SERA	REACTION AFTER HEATING FOR THIRTY MINUTES AT 56° C	REACTION AFTER HEATING FOR FIVE MINUTES AT 62° C
23	++++	++++
1	++++	+++
1	++++	++
1	++++	+
1	++++	±
1	+++	++++
2	+++	+++
4	+++	++
1	+++	±
1	++	++++
2	++	++
1	++	+
1	++	±
1	+	+
1	±	±
1	±	—
1	—	+
216	—	—

(5 per cent) showing less sensitive reactions than the control, while the other 3 (1 per cent) gave more sensitive reactions. Considering the relatively large number of undersensitive reactions shown by the serum fractions heated for five minutes at 62°C , this heating period does not appear to be satisfactory for the test.

Experiment 4—A heating treatment of three minutes at 65°C was then tried employing 35 sera and controlled in a manner similar to that used in the previous experiments. Undersensitive results as compared with the control were obtained with 20 per cent of the sera while only one serum fraction showed a more sensitive reaction than that of the control.

Experiment 5—An experiment was carried out to determine the relative sensitiveness of a serum heated at 56°C for varying time periods. Several portions of a strongly positive pooled serum were placed in a water bath at 56°C , portions being removed at successive intervals and tested by means of the quantitative procedure. The length of the heating periods ranged from ten minutes to five hours. The results with this pooled serum are plotted in Fig 1, in which the ordinates represent serum sensitiveness in Kahn units, and the abscissae represent the heating period in minutes. The sensitiveness increased consistently until the heating period reached forty minutes, and remained practically the same until the seventy minute period was reached. Longer heating periods reduced the sensitiveness, but even the maximum heating period (five hours) did not reduce the sensitiveness to that of the unheated serum. The results obtained with this serum confirmed the general relation between the sensitiveness and the heating period brought out in Kahn's early studies.

Experiment 6—Employing a method similar to that of the previous experiment, a serum was heated at 60°C , portions being withdrawn at intervals ranging from two to one hundred and thirty minutes and tested by means of the quantitative procedure. The results of this experiment show that a serum heated at 60°C reaches its maximum sensitiveness in a comparatively short time, in this case between two and fourteen minutes. Portions of this serum heated for longer periods showed a fall in sensitiveness, reaching a level below that of the unheated serum when the heating period reached eighty minutes.

CONCLUSIONS

- 1 Heating of serum in the Kahn test for ten minutes at 60°C gives practically the same results as the regular heating period of thirty minutes at 56°C .
- 2 Serum heated at temperatures of 62°C or more is not satisfactory for use in the Kahn test.
- 3 Serum heated at 56°C reaches maximum sensitiveness in from thirty to seventy minutes while serum heated at 60°C reaches maximum sensitiveness in from two to twelve minutes.

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MÜLLER'S CONGLOBATION REACTION FOR THE DIAGNOSIS OF SYPHILIS^{*}

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IN PRESENTING this paper on Muller's conglobation reaction, we are simply attempting to make a comparison between this test, which we believe is little known in this country, and the well-known Kahn test. So much literature has appeared in the journals in recent years concerning the relative merits of flocculation tests as compared with the complement-fixation tests that it has become definitely apparent that the flocculation test is rapidly supplanting the complement-fixation reaction as a routine laboratory measure. Few laboratories have adopted the newer test without first having convinced themselves of its practical value by running long series of parallel tests with the complement-fixation system in use. The choice of the flocculation test to be used is a matter to be determined in the same manner, and it was with this view in mind that the series presented was undertaken. It is sufficient to say here that the only differences in the various flocculation tests so far brought forth, and they are legion, are the variations in the concentrations of the complex reagents under study. The physical and biologic principles involved are identical in all cases.

The technic presented here is the same as used by Muller himself in Vienna, the only variation being in the matter of readings. Muller makes a reading after the incubation period and again after the racks have stood at room temperature for eighteen hours, and averages the two. We made one reading only at the end of the total period, which seemed preferable for purposes of comparison. The readings of the three tubes were averaged to give the final report of the strength of reaction.

The antigen is prepared by adding one part of ox heart (removed from the apex of the heart, all free fat cut away, and macerated in a food chopper) to thirty parts of 96 per cent alcohol. The heart muscle is added slowly, a small bit at a time, while the beaker containing the alcohol is kept in constant motion to obtain as much extraction as possible. The flask is stoppered with a rubber cork and placed in the incubator for eighteen hours at 56° C and is shaken from time to time during the day. After the incubation period has elapsed, the flask is allowed to stand at room temperature for forty-eight hours. The contents are now carefully filtered, placed in a brown bottle and allowed to stand at room temperature for at least two months. At the end of two weeks if there is any visible sediment on the bottom of the bottle, the contents must be again filtered. When the antigen is fully matured, it is cholesterinized by adding 7½ c c of ½ per cent cholesterol in alcohol to each 30 c c of the extract. This amount (approximately 38 c c) is evaporated to

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8 c.c. in a boiling water bath, this extract constituting the finished product. Such a process is not so laborious as it would seem if one has been accustomed to making the similar alcoholic extract used in the complement fixation tests. In any case, it is advisable to purchase the antigen, that is, the final extract, from a reliable maker. It may be obtained from Scheering in Berlin, but we are not advised if it is possible to obtain it in this country.

The only difficult phase of the test is in making the dilution of the standard extract with saline solution for use in the test proper. The dilution is carried out as follows. Eight c.c. of the standard extract are placed in a test tube and incubated in a water bath at 56° C. for one half hour. The heating causes the resolution of any flaky particles. Five c.c. of saline solution (0.9 per cent) are placed in a small glass bowl about 45 mm. in diameter (No. 1), and 50 c.c. of saline placed in a second bowl of the same size (No. 2). The salt solution in both glass bowls must be brought to a temperature of 17° C. accurate to within half a degree. When this has been done the contents of the test tube previously incubated for one half hour are poured into glass bowl No. 1 and as quickly as is possible the contents of glass bowl No. 2 are added to this mixture. The colloidal solution thus obtained is poured into test tubes 18 to 20 mm. in diameter, sealed with India rubber stoppers and placed in an incubator (56° C.) for twenty-four hours. It is then ready for use. In order to obtain a good conglobation reagent the directions given here should be strictly adhered to, especially in regard to the temperature of the salt solution, period of maturing, size and sealing of the glass vessels, temperature of the water bath, incubator, etc.

These physical data have been very carefully worked out by Muller to ensure an optimum dispersion of the colloidal antigen.

The Test Proper.—The sera are inactivated for one half hour in a water bath at 56° C. Three small, thoroughly cleaned test tubes (Widal tubes) with an inner diameter of about 8 mm. are set up for each case to be tested. To these are added 0.15 c.c. (3 drops), 0.2 c.c. (4 drops) and 0.25 c.c. (5 drops) of the sera, respectively. The antigen in the test tubes is shaken once or twice to ensure that any flocculi are resuspended and to each tube is added 0.5 c.c. of this matured antigen. The racks are shaken by hand for a few seconds and then placed in the incubator (37° C.) for six to eight hours when they are removed and left to stand at room temperature for from nine to fifteen hours before the final reading is made.

In the case of a positive reaction one obtains a fine suspended globular compound of a white or yellowish white gelatinous appearance in the middle of the cylinder of fluid, usually with a more dense rather darker colored center, and surrounded by a soft veil like covering. If the antigen has not been properly prepared, the conglobation has often a more dense crumbly appearance. The various degrees of strength of reaction are read according to the completeness of the formation of the "conglobat" and to the amount of flocculation visible. The occurrence of nonspecific reactions is extremely rare. When they do occur, a dense precipitate of large flakes is visible which are so different in appearance from the specific reaction that they cannot be mis-

taken It is characteristic of nonspecific reactions that the lower serum doses often react more strongly than the higher dose, this being in the nature of a zone phenomenon

Analysis of Results—In the whole series investigated, the two tests were in absolute accordance in the frankly negative and frankly positive cases These we shall not consider further Table I shows the relative strengths of reaction of the two tests in a series of selected, untreated, "border-line" cases

TABLE I

CASE	KAHN				MULLER			
	TUBE NO 1	TUBE NO 2	TUBE NO 3	RESULT	TUBE NO 1	TUBE NO 2	TUBE NO 3	RESULT
1	—	±	+++	±	—	—	±	—
2	—	++	+++	+	++	++	++	++
3	—	±	+++	+	+	+	+	+
4	—	±	+++	+	—	—	±	—
5	—	±	++	±	—	—	±	—
6	—	±	+++	+	+	+	+	+
7	±	+	+++	+	++	++	++	++
8	—	—	++++	+	+	++	+++	++
9	—	±	+++	+	—	++	++	+
10	—	±	++	±	—	++	++	+
11	—	±	+++	+	+	++	+++	++
12	—	±	++	±	—	+	++	+
13	—	+	++	+	+	++	++	++
14	—	+	++	+	+	++	++	++
15	±	+	++	+	+	+	+	+
16	—	+	++++	+	+	++	+++	++
17	±	+	++	+	+	++	+++	++
18	—	±	+++	+	+	++	++	++
19	—	+	++	+	+	++	++	++
20	±	++	++	+	+	++	+++	++
21	—	+	+++	+	++	+++	+++	+++
22	—	±	+	±	+	+	+	+
23	±	+	+++	+	+	++	++	++
24	—	±	+	±	—	±	+	±
25	—	±	+++	+	+	+	+	+

The cases in Table I were selected at random from the series to present the types of reaction met with in the weakly positive cases In this class of cases, for the whole series the Muller test gave a stronger reaction in 80 per cent of the sera examined, complete agreement in 15 per cent, and disagreement in 5 per cent The latter are composed principally of cases which gave a one-plus or a plus-minus reaction to the Kahn test and a negative reaction to the Muller From this it would appear that the Kahn test was more sensitive in these faintly positive reactions, but it must be pointed out, however, that the Muller test is very much easier to read and especially where there is only the faintest amount of flocculation present This factor must be taken into account, in spite of the fact that the Kahn tests were all read by an expert with a very considerable experience Again, the Kahn tests showed little reaction in the first two tubes in the tests and quite a strong reaction in the third tube, which brought the average of the three tubes up to a much higher level than would have been the case if each individual tube was taken as a criterion of its own sensitiveness This was not so in the Muller test, a gradual increase in the degree of the reaction being the general rule, which shows

quite conclusively that the Muller antigen is the more sensitive of the two. Coupled with this fact we have already seen that in no case did the Muller test give a positive reaction where the Kahn test remained negative. The converse was true in 5 per cent of the 300 cases examined. This would be interpreted by us as an additional indication that the Muller antigen is even more sensitive than the Kahn. That it is not too sensitive is borne out by the fact that in no case did we get a 'false positive' reaction.

Table II is composed of cases selected in the same manner as those in Table I and represents reactions of a stronger nature. Here, too, it will be seen that the Muller test gave a stronger reaction than the Kahn. In 85 per cent of the cases in this group the Muller test gave a stronger reaction than the Kahn test, in 12 per cent there was agreement and in 3 per cent the Kahn test gave a stronger reaction than did the Muller test. Although the strength of reaction in cases of this type is not so significant as that in Table I it is additional evidence of the sensitiveness of the Muller antigen.

TABLE II

CASE	KAHN				MULLER			
	TUBE NO 1	TUBE NO 2	TUBE NO 3	RESULT	TUBE NO 1	TUBE NO 2	TUBE NO 3	RESULT
1	+++	+++	+++	+++	+++	+++	+++	+++
2	+	+++	++++	+++	++++	++++	++++	++++
3	++	+++	+++	+++	++++	++++	++++	++++
4	++	+++	++++	+++	+++	+++	+++	+++
5	++	++	+++	++	++	++	++++	+++
6	++	++	++++	+++	+++	+++	+++	+++
7	++	+++	++++	+++	++	+++	++++	+++
8	++++	+++	++	+++	+++	+++	+++	+++
9	+	+++	++++	+++	++	+++	++++	+++
10	+++	+++	+++	+++	++++	++++	++++	++++
11	++	+++	++++	+++	++	+++	++++	+++
12	++	+++	+++	+++	+++	++++	++++	++++
13	++++	+++	++	+++	+++	+++	+++	+++
14	+	++	++++	++	+++	+++	+++	+++
15	++	++	++++	+++	++	++	+++	++
16	++++	++	++	+++	+	++	+++	++
17	-	+++	++++	++	++	+++	++++	+++
18	-	++	++++	++	+	++	+++	++
19	±	+++	++++	++	++++	++++	++++	++++
20	++	+++	+++	+++	++	++	++	++
21	+	+++	+++	++	+	++	+++	++
22	-	++	++++	++	++	++	++	++
23	++	+++	++++	+++	+++	++++	++++	++++
24	-	+++	+++	++	++	++	++	++
25	-	+++	+++	++	++	+++	++++	+++

In the 3 per cent of cases in this group which gave a stronger reaction with the Kahn test than with the Muller test, 2 per cent of the Kahn reactions were of the type shown by No 13, that is, sera which gave a stronger reaction in the higher dilutions than in the lower. Although this type of serum is not often met with, it is significant that the Kahn test gave a descending degree of reaction to the higher dilutions where the Muller gave an ascending degree, as was the case in the weak positives.

We were not in a position to make a detailed study of treated cases and will, therefore, not attempt to analyze results of those we have done in this series.

CONCLUSIONS

1 The technic of the Muller reaction is as simple as that of the Kahn, with the exception of the preparation of the antigen, which is both more complicated and time consuming

2 The Muller reaction is considerably easier to read than the Kahn. This reduces the personal element of error and makes for more uniform interpretation of results

3 The Muller reaction is more sensitive than the Kahn reaction in weakly positive cases and gives a stronger reaction in cases with a higher degree of positiveness

4 Although the antigen is more sensitive than the Kahn antigen we did not encounter any "false positives," which are easily detected by the marked differences in the flocculi

The tests were carried out in the Public Health Laboratory of Nova Scotia under the direction of Dr D J MacKenzie. The Kahn tests were done by Miss M L Low, chief technician in the same laboratory. My thanks are due to them both.

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A MICROSCOPIC SLIDE PRECIPITATION TEST FOR SYPHILIS WITH UNHEATED SERUM*

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THE microscopic slide precipitation test for syphilis with unheated serum, described below, gives results almost identical with those of the test with heated serum and is more sensitive than the Wassermann test of heated serum with the same antigen.

The precipitation test with unheated serum based upon the principles of the microscopic slide precipitation test for syphilis with heated serum,^{1, 2} like the latter, is a thoroughly satisfactory, simple test for use in the diagnosis of syphilis.

THE MICROSCOPIC SLIDE PRECIPITATION TEST FOR SYPHILIS WITH UNHEATED SERUM

Into each of 36 rings on three glass slides, 0.05 cc of unheated serum to be tested is delivered from a pipette (the tip of the pipette is placed in the center of the ring and the serum allowed to run out. Eighteen sera in duplicate are pipetted).

After all the sera are pipetted, one drop of 5 per cent salt solution (about 0.015 cc) is allowed to fall from a capillary pipette into the serum in each ring.

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The slides (in a holder) are rotated on a flat surface with moderate vigor for one minute

Into one half of the serum salt solution mixtures, one small drop (about 0.007 cc) of sensitive antigen emulsion is allowed to fall from a capillary pipette. Into each of the other serum salt solution mixtures, one small drop (about 0.0065 cc) of very sensitive antigen emulsion is allowed to fall from a capillary pipette.

The slides in the holder are rotated on a flat surface with moderate vigor for four minutes.

The results are examined at once through the microscope at a magnification of about 100 times (low power 16 mm objective, eyepiece 10X or 12½X) with the light cut down as in studying urinary sediments and recorded in terms of pluses according to the degree of clumping and size of clumps.

MATERIALS FOR THE MICROSCOPIC SLIDE PRECIPITATION TEST FOR SYPHILIS WITH UNHEATED SERUM

Glassware—Microscopic slides 2 by 3 inches as purchased are rubbed on both sides with bon ami paste (prepared by allowing a cake of bon ami to remain in sufficient warm water to cover it for twelve hours or more. The paste keeps well, but may require slight dilution with water from time to time). As soon as the paste is dry (in about five minutes) it is completely removed from the slide with a soft muslin cloth. For convenience the slides covered with paste may be stuck to each other, allowed to dry and cleaned at any time. Upon the clean slides, 12 paraffin rings each with an inside diameter of 13 mm are mounted. On slides so cleaned serum spreads freely. After use, the slides may be washed in hot water and prepared again as outlined above.

Instrument for Making Paraffin Rings—This is essentially the instrument proposed by Green.⁵ A piece of soft iron wire (No. 28) 14 cm in length is wound twice tightly around a test tube about 13½ mm in outside diameter, forming a double loop and leaving a double shaft about an inch in length. The two shafts are then twisted together to within a quarter of an inch of the free end. After removing the looped wire from the test tube a piece of linen thread (No. 12) about a yard long is started from the free end of the shaft after being fastened here by a single twist of the two free ends. Three long turns are made, reaching the loop which is then tightly wound with the thread, the winding is continued up the shaft to the free end where it is fastened between the two ends of the wire by twisting them. The loop is then bent at right angles to the shaft. It is then reshaped by working it against the bottom of the test tube mentioned above. The shaft is then inserted into the handle of a teasing needle or into a straight hemostatic forceps.

The paraffin rings are made by dipping the instrument into smoking paraffin (about 120° C), drawing quickly at one point and transferring the remainder to the glass slide.

Pipettes—The pipettes used for delivering the serum are the ordinary 1 cc pipettes, graduated in 0.01 cc. The pipette for the antigen dilution is a capillary pipette made from glass tubing 8 to 10 mm in diameter with the tube

about $\frac{3}{5}$ mm in diameter, delivering a drop equal to 0.0065 to 0.0075 c.c. (77 to 67 drops per $\frac{1}{2}$ c.c.) The pipette for the 5 per cent salt solution is a similar capillary pipette with the tube about $\frac{3}{4}$ mm in diameter delivering a drop equal to about 0.015 c.c. (33 drops per $\frac{1}{2}$ c.c.)

Salt Solution—Five per cent sodium chloride (c.p. or reagent, Merek) solution is used in the test. This is made with distilled water (Distillata, Cleveland) having a P_H of 5.4 to 6.0, such water gives very light purplish-red to medium purplish-red color with chlorphenol red indicator (La Motte). Distilled water having a P_H of 5.2 or less, gives a yellow color with this indicator and is not as satisfactory.

Antigen—The antigen is a lipid obtained by precipitation in acetone at 50° C. to 37° C. of concentrated absolute alcohol extract of beef heart muscle powder (Difco). The details of its preparation are given in a previous report.²

Antigen Emulsions—The antigen emulsions have the following formulas

1 VERY SENSITIVE ANTIGEN EMULSION	2 SENSITIVE ANTIGEN EMULSION
0.85 c.c. Distilled water (P_H 5.4 to 6.0)	0.85 c.c. Distilled water (P_H 5.4 to 6.0)
1.25 c.c. of 1 per cent Cholesterol (Pfanderstichle) in absolute ethyl alcohol (99+ per cent) (Prepared in about forty five minutes by placing in an oven at 50° to 56° C. and shaking gently a few minutes at fifteen minute intervals)	0.95 c.c. of 1 per cent Cholesterol (Pfanderstichle) in absolute ethyl alcohol (99+ per cent)
0.1 c.c. Antigen	0.1 c.c. Antigen
2.2 c.c. of 0.85 per cent Sodium Chloride (c.p. or Reagent, Merek) solution (made with distilled water P_H 5.4 to 6.0)	2.5 c.c. of 0.85 per cent Sodium Chloride (c.p. or Reagent, Merek) solution (made with distilled water P_H 5.4 to 6.0)

These emulsions are prepared as follows

Into a one ounce bottle 0.85 c.c. of distilled water is pipetted

The bottle is held at an angle and the 1 per cent cholesterol in absolute ethyl alcohol (99+ per cent) is allowed to run along the side of the neck of the bottle

The bottle is gently rotated from the neck for twenty seconds

The bottle is held at an angle again, and 0.1 c.c. of antigen is pipetted against the side of the neck from a 0.2 c.c. pipette (graduated in thousandths)

The bottle is promptly stoppered with a cork and shaken fairly vigorously (the fluid thrown from bottom to cork and back) for one minute

Lastly, the 0.85 per cent sodium chloride solution is allowed to run in quite rapidly, the bottle is stoppered again and shaken as previously for one minute

The emulsions, when examined through the microscope, at a magnification of about 100 times, show numerous very fine particles but no clumps whatever. The emulsions when tested on heated syphilitic sera show a steady increase in sensitivity for one-half hour after preparation, a maximum sensitivity from one-half hour to six hours after preparation followed by a slight steady decline in antigenic power. They are, therefore, used in tests on unheated sera any time from one half to six hours after preparation

TABLE I

COMPARISON OF VERY SENSITIVE UNHEATED SERUM SLIDE PRECIPITATION TESTS WITH VERY SENSITIVE HEATED SERUM SLIDE PRECIPITATION TESTS
AND WITH VERY SENSITIVE HEATED SERUM WASSERMANN TESTS ALL WITH THE SAME ANTIGEN

	VERY SENSITIVE UNHEATED SERUM AND VERY SENSITIVE HEATED SERUM SLIDE TESTS				VERY SENSITIVE UNHEATED SERUM SLIDE TESTS AND VERY SENSITIVE HEATED SERUM WASSERMANN TESTS			
	CONSENT Tests Cent	RELATIVE AGREEMENT Tests Cent	RELATIVE AGREEMENT Tests Cent	DISAGREEMENT Per Cent	TOTAL TESTS	CONSENT Tests Cent	RELATIVE AGREEMENT Tests Cent	RELATIVE AGREEMENT Tests Cent
Tests with P _n of distilled water used 5.4 to 0.6	400 93.33	33 0.29	323 99.62	2 (neg unheated, pos heated)	525	121 87.34	41 8.51	162 95.81
Tests with no P _n control of wa- ter			6 (neg unheated, pos heated)	0.38				20
Tests with P _n 6.4 to 0.6 and with no P _n control of water	1267 89.79	137 0.71	1404 99.50	1 (pos unheated, neg heated)	1411	1139 83.43	169 12.39	28 (neg unheated, pos Wassermann)
	1757 90.76	170 8.78	1927 99.53	8 (neg unheated, pos heated)	1936	1759 84.44	210 11.38	43 (neg Wassermann, pos unheated)
				1 (pos unheated, neg heated)				29 (neg unheated, pos Wassermann)
				0.47				95.83
								77
								1846

Wassermann Test Cleveland method

Antigen containing 0.3 per cent cholesterol

Positive Reaction ++++ and ++

Doubtful Reaction + and ±

Agreement = positive negative or doubtful by both methods

Relative Agreement = positive or negative by one method and doubtful by the other

Disagreement = positive by one method and negative by the other and vice versa.

TABLE III
COMPARISON OF UNHEATED SERUM SLIDE PRECIPITATION TESTS AND DEBRINATED FINGER BLOOD PRECIPITATION TESTS

	SENSITIVE ANTIGEN EMULSION						VERY SENSITIVE ANTIGEN EMULSION					
	AGREEMENT		RELATIVE AGREEMENT		DISAGREEMENT		AGREEMENT AND RELATIVE AGREEMENT		RELATIVE AGREEMENT		DISAGREEMENT	
	Per Tests	Cent	Per Tests	Cent	Per Tests	Cent	Per Tests	Cent	Per Tests	Cent	Per Tests	Cent
Tests with 1/2 of distilled water used	82	82	18	18	0	0	100	100	0	0	100	100
5.4 to 6.0	82	82	18	18	0	0	100	100	0	0	100	100

(For illustrations of glassware, holders and results, see articles ^{1, 4, 5})

Tables I and II show a comparison of the results of the precipitation test of unheated serum with other tests for syphilis (Approximately 20 per cent of the sera tested were from syphilitic patients)

The tables show that the microscopie slide precipitation test for syphilis with unheated serum gives results almost identical with those of the precipitation test with heated serum and gives more positive results than the Wasserman test (heated serum) with the same antigen

Table III shows that the unheated serum precipitation test gives results almost identical with those of the defibrinated finger blood precipitation test ⁴

COMMENT

The precipitation test for syphilis with unheated serum, although based upon the principles of the test with heated serum, differs from the latter in a number of ways

In the first place, a quantity of salt solution is added to unheated serum to increase its agglutinating power to equal that obtained by heating serum at 56° C for one half hour

In unheated serum tests, the quantity of electrolytes present must be carefully controlled, since relatively little more or less than the optimum quantity changes the result appreciably. Heated serum, on the other hand, is much less affected by the addition of electrolytes. In testing unheated sera, therefore, it is of the greatest importance to know the nature of the distilled water used in the preparation of the salt solution and antigen emulsions to be added. When distilled water containing relatively many acid ions (P_H 5 and less) is used for the test preparations, 0.015 c.c. of 3 per cent sodium chloride solution is the quantity to add to 0.05 c.c. of serum for maximum sensitivity. When distilled water of P_H 5.4 to 6.0 is employed, 0.015 c.c. of 5 per cent sodium chloride solution is the amount to add for maximum sensitivity.

Results with distilled water of P_H 5.4 to 6.0 in the preparation of the salt solution and antigen emulsions have been *definitely better than those with water of P_H 5.0 and less*

In the two tests, no appreciable difference in results occurs when antigen emulsions are used one-half hour to six hours after preparation. In the unheated serum test with antigen emulsions over six hours old a slight steady decline in sensitivity of results occurs. In the heated serum tests, on the other hand the same emulsions give equally sensitive results for at least forty-eight hours after preparation.

With proper precautions, as noted above, the microscopie slide precipitation test for syphilis with unheated serum gives results almost identical with those of the test with heated serum.

CONCLUSION

The microscopie slide precipitation test for syphilis with unheated serum described above, gives results almost identical with those of the test with heated serum and is more sensitive than the Wassermann test with the same antigen.

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- 3 Green, G Paraffin Rings on Microscopic Slides *Am J Pub Health* 15 651 1925
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- 5 Kline, B S, and Young A M A Microscopic Slide Precipitation Test for Syphilis (Preliminary Report), *J A M A* 86 928, March 27, 1926

A METHOD FOR THE DETERMINATION OF THE CALCIUM CONTENT OF PUS*

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IN CONNECTION with some work undertaken here it was necessary to determine the calcium content of small quantities of pus. Although methods for this are available it was thought that a simple procedure requiring no special equipment would be of value. The method suggested here requires only apparatus found in the ordinary clinical or chemical laboratory and is easily carried out. Although tested by us only on pus and blood serum, we see no reason why it might not be applicable to any biologic material.

METHOD

The material to be analyzed is measured by means of an accurately calibrated pipette into a pyrex centrifuge tube of 15 cc capacity, or else placed directly in a similar tared tube and weighed. To this is added 1 or 2 cc concentrated HNO_3 , and several drops concentrated H_2O . Merck's Superoxol was used by us. A very small piece of quartz or fused silica is placed in the tube to prevent bumping and loss of material by spattering. When working with pus it was also necessary to add an antifoaming substance before starting the heating. We found the addition of one drop of oetic alcohol to be satisfactory for this purpose. The mixture is then digested by heating cautiously over a flame. More HNO_3 and H_2O are added from time to time as these substances boil off in the heating. The material can be heated practically to dryness since charring does not interfere. The charred material is oxidized easily by further addition of HNO_3 and H_2O and continued heating. When all organic matter has been oxidized the contents of the tube are heated almost to dryness in order to remove most of the HNO_3 . Water is then added and the acid neutralized by dilute NH_4OH using phenol red as indicator. If the quantity of pus is so small that only a small amount of calcium is present, a known amount of calcium is added at this point. One cc of a solution containing 0.1 mg calcium may be used for this purpose. The volume in the tube

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is now made up to 4 cc. The procedure from now on is the same as that of the ordinary Kramer-Tisdall method¹. Add 1 cc of saturated $(\text{NH}_4)_2\text{C}_2\text{O}_4$ and allow the mixture to stand for one half hour. Centrifuge for five minutes and then pour off the supernatant liquid being careful not to lose any of the precipitate. The tube is then allowed to drain upside down for five minutes and the precipitate is then washed with 3 cc of 2 per cent NH_4OH . Centrifuge again for five minutes and again pour off the supernatant liquid and drain the tube upside down for five minutes. In the draining, the tube is allowed to stand on a soft piece of gauze or filter paper to absorb the liquid. After the draining, the lip of the tube is wiped to remove any liquid that might be adherent. Add 2 cc of $\text{N H}_2\text{SO}_4$ and titrate with 0.005 N KMnO_4 at a temperature of 70°C .

COMMENT

The results obtained by this method are given in Table I. For these analyses both blood serum and pus were used. In the case of the blood serum, the results are compared with those gotten by the ordinary Kramer-Tisdall procedure. It is to be noted that they agree quite closely when the quantity of calcium is

TABLE I

MATERIAL	AMOUNT GM	AMOUNT Ca ADDED MG	AMOUNT Ca FOUND MG	Ca CONTENT MG PER 100 GM	METHOD USED	VARIATION
Serum I	1.989	0.0	0.218	11.0	K T ¹	0.0
"	0.510	0.098	0.153	10.8	W A ¹	-0.2
"	0.538	0.0	0.068	12.6	K T	+1.6
"	0.494	0.098	0.154	11.3	K T	+0.3
"	0.517	0.0	0.095	18.3	W A	+7.3
"	1.000	0.0	0.130	13.0	W A	+2.0
Serum II	1.983	0.0	0.209	10.5	K T	0.0
"	0.516	0.098	0.152	10.5	W A	0.0
"	1.020	0.0	0.109	10.7	W A	+0.2
"	0.516	0.0	0.063	12.2	W A	+1.7
Serum III	1.014	0.0	0.116	11.4	W A	+0.1*
Pus (A) ²	1.072	0.0	0.114	10.7	Inc ³	0.0
"	2.026	0.0	0.212	10.5	W A	-0.2
"	1.067	0.0	0.111	10.4	W A	-0.3
Pus (B)	1.069	0.0	0.112	10.5	Inc ³	0.0
"	1.052	0.0	0.102	9.7	W A	-0.8

* A 2 cc sample of this serum gave 11.3 mg per 100 gm serum by the Kramer-Tisdall method. The calculations were lost and are therefore not given here.

¹K-T represents Kramer-Tisdall. W-A represents wet ashing, the procedure suggested here.

²In the case of the pus samples results obtained by our procedure are compared with those gotten by incineration. Both samples were obtained from empyema cases.

properly adjusted. Attention is drawn to the fact that when the amount of calcium present is very small the determination by precipitation and subsequent titration yields inaccurate results. But if a small amount of calcium is added as suggested in this method, errors are eliminated and our results check with those obtained by other methods. It is of course, understood that the amount of calcium added should not be too great in proportion to that present. From our own results it seems safe to add as much as twice the quantity already present without introducing any doubt as to the accuracy of the findings. If the amount added is greater than that, one might question the validity of this procedure.

In the case of the pus samples, there were only two instances where enough material was available for comparison of the results obtained by this technique with those obtained by incineration. These, however, show a fair agreement. The other values given for the calcium content of pus (Table II) agree quite closely with those given by Friesner and Rosen for the calcium content of pus obtained from various tissues.

TABLE II

CALCIUM CONTENT OF PUS OF SOFT TISSUE ORIGIN AS DETERMINED BY THE PROCEDURE SUGGESTED

SOURCE	AMOUNT GM	AMOUNT Ca ADDED MG	AMOUNT Ca FOUND MG	Ca CONTENT MG PER 100 GM
Hip ¹	1.915	0.0	0.224	11.1
"	1.220	0.0	0.126	10.3
Ear ²	0.491	0.098	0.153	11.2
"	0.355	0.098	0.134	10.1
Leg ³	0.654	0.098	0.164	10.1

¹The samples of pus from the hip are from the same individual but taken at different times.

²The samples from the ear are also from the same individual but represent different ears.

³This represents a serous exudate taken from a severe burn.

The authors are indebted to Dr. Philip Romonek, of the Department of Otorhinolaryngology, for suggesting the problem and for aid in collection of some of the samples of pus.

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- 2 Friesner Isidore and Rosen, Samuel. A New Aid in the Diagnosis of Mastoiditis. Arch. Otolaryng. 7: 317, 1928.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

ACIDOSIS Preparation of Sodium Bicarbonate for Intraperitoneal Use, Shohl, A. T.
Am J Dis Child 38 953, 1929

One hundred cubic centimeters of 1.3 per cent sodium bicarbonate (isotonic) is placed in a 200 cc. pyrex flask. We have used pressure bottles such as are generally employed for citrate of magnesia, or pyrex nursing bottles have been used with good success. A few drops of phenol red are introduced. Carbon dioxide from a tank is passed into the solution until the color of the solution corresponds to that of the standard buffer solution at P_H 7.2 (this contains 8 parts of 15 molar sodium phosphate and 2 parts of sodium biphosphate) which contains the same amount of indicator. A rubber stopper is inserted tightly. This is covered with two layers of tin foil or heavy paper. A string is tied tightly around the neck of the flask to hold the stopper in place. The flask is then autoclaved at 15 pounds (6.8 kg.) pressure for fifteen minutes. Occasionally, a stopper will leak or a flask will break in the autoclave. The temperature should be reduced gradually. The solution heated becomes more alkaline or if chilled in the ice box more acid, owing to the effect of temperature on exchange of carbon dioxide in and out of the solution. On return to room temperature, its original color should return. Such a solution may be kept at least two weeks without alteration.

TISSUE Methods for the Histologic Study of Normal and Diseased Bone, Jaffe, H. L.
Arch Path 8 817, 1929

This paper, which is in the nature of a general review, presents a comprehensive review of methods too long and too detailed to be satisfactorily abstracted but well repaying perusal.

KNEE JOINT EFFUSIONS Erythroblasts and Myelocytes in Traumatic Knee Joint Effusions, Kling, D. H. *Am J Surg* 7 824, 1929

In intraarticular fractures bone marrow elements will reach the knee joint. Erythroblasts and myelocytes signify an intraarticular fracture, the presence of fat indicates severe injuries.

Complement Preservation of, Ruffner, E. *Z Immunitat* 60 166, 1929

Ruffner believes 10 per cent solution of sodium acetate containing 4 per cent boric acid to be the best, the next best being 10 per cent sodium chloride with 4 per cent boric acid and diluted with an equal volume of glycerol.

RETICULOCYTES A Simple Method of Obtaining Permanent Preparations, Wright, J. H.
Glasgow M J 3 292, 1929

A drop of saturated aqueous solution of cresyl blue, about one sixteenth of an inch in diameter, is put on the ear with the broad end of a needle. The skin is punctured through the drop, and when the mixture is about one eighth of an inch in diameter films are made or cover slips in the ordinary way. These are allowed to dry in air for three to four minutes, and are then stained with Leishman.

UROBILIN Determination of, in Urine Tixier L. Bull d sc. pharmacol 36 555, 1929

To 20 cc of urine add 5 cc of normal sodium hydroxide and 25 cc of 10 per cent barium chloride. A normal urine containing 0.45 gm of urobilin per 100 will give a filtrate the same color as a 0.004 per cent solution of potassium bichromate.

SYPHILIS A Rapid Precipitation Test Rosenthal, L. Proc Soc Exper Biol & Med 27 61 1929

Inactivated serum is used.

The antigen is prepared by adding 2 per cent solution of cholesterol in acetone to an equal volume of alcoholic beef heart extract. This extract is obtained by adding 5 cc of alcohol (95 per cent) for every gram of beef heart muscle powder from which the ether soluble substances were previously removed by ether extraction.

BLOOD TRANSFUSION In Diseases of Infants and Children, Krahulik L. and Koch L. A. Am J Dis Child 39 34 1930

The authors determine the dose by the formula:

$$\frac{\text{Weight}}{\text{Height} + 40} \times 500 = \text{amount to be given.}$$

PNEUMOCOCCUS Nutrient Medium With Liver Extract Quiroga R. Rev Soc Argent de biol 6 4 1928

Extract beef liver with 2 parts of 0.5 per cent NaCl solution for two hours at 45° C heat slowly to 60 C; filter through paper and then through a sterilized cylinder. Between 2 and 5 per cent are added to broth of P_H 8.4.

TISSUE Hematein Stain Kornhauser S. I. Stain Tech 5 13 1930

Paraffin or celloidin sections of Boun or Zeaker formal material are run down to water and stained about five minutes in Mayer's hemalum (0.5 gm hematein ground up in a glass mortar with 10 cc 95 per cent alcohol and added to 500 cc of 5 per cent aqueous solution potassium alum). Rinse 1 to 3 seconds in tap water. Dip 1 to 3 seconds in eosin B (1 part 0.5 per cent solution in 20 per cent alcohol added to 2 parts distilled water, filtered from time to time). Wash several minutes in running water or in several changes of tap water. Dehydrate and mount, with unattached celloidin sections this may be done by running up to 95 per cent alcohol spreading on slide blotting, wetting with absolute alcohol draining and mounting in cupral.

TUBERCLE BACILLUS Comparison of Petroff's and Petraguani's Methods for Primary Culture Terzani A. Gior di clin med 11 7 1930

Preparation of the Petraguani Medium

In a 1 liter beaker place 150 cc of fresh milk, 6 gm of potato chlorophyll (sic) 1 gm of peptone and fragments of whole potato about the size of an egg. The beaker is placed in a water bath and heated to boiling. The mixture should be gently agitated until the milk, peptone, and chlorophyll have formed a sort of curd (five to ten minutes) after which the mixture is left in the boiling water bath for one hour. It is then removed and when the mixture has cooled to below 60 (sic) 4 whole eggs and the yolk of a fifth are added with thorough mixing under aseptic precautions. The homogeneous mixture is then filtered through sterile gauze into a sterile 500 cc Erlenmeyer flask. Twelve ounces of neutral glycerin and 10 ounces of 2 per cent aqueous solution of malachite green are then added.

The medium is now distributed in tubes which are slanted in a coagulator at 85° C for twenty five minutes.

Preparation of Sputum for Culture

A few centimeters of sputum are placed in a sterile beaker and made homogeneous

by agitation with sterile shot with the addition of several drops of litmus solution and sufficient 4 per cent sodium hydroxide solution (equal volumes or more in accordance with the original consistency of the sputum) The mixture may be placed in the incubator at 37° C though this is not essential

The reaction is then neutralized by the addition of 10 per cent hydrochloric acid This must be carefully done with continuous agitation of the mixture as the addition of an excess of acid will vitiate the results

Inoculation About 0.5 cc of the treated specimen is planted in each of not less than 4 or 5 tubes The plugs are pushed in, cut off level with the top of the tube, and well paraffined If necessary this should be repeated to prevent drying out The cultures are incubated in a horizontal position so that the liquid inoculum is distributed over the surface of the medium They should not be disturbed for 4 or 7 days Colonies are punctiform and light yellow in color and stand out distinctly against a green background Later they become more or less confluent

TUBERCULOSIS A Biologic Reaction in the Urine of the Tuberculous, Franco, E Pat e Clin di Tuberc 2 849, 1929

The test in question is that proposed by Piazza

Preparation of the antigen

A rabbit (1500 gm) is immunized with four intraperitoneal injections of Koch's old tuberculin

The injections are given ten days apart in the following amounts 2 cc, 4 cc, 6 cc, and 10 cc

The animal is bled with sterile precautions fifteen days after the last injection

The test 5 cc of urine, preferably sterile, and rendered clear and limpid by filtration are placed in each of two sterile tubes To one is added 0.5 cc of the immune serum The other tube is the control Both tubes are incubated at 37° C for twenty four hours

There are two phases to the reaction The first, read after incubation, is the precipitating phase and consists in the appearance of a precipitate in the test tube and not in the control If precipitation appears in both tubes, the reading is positive only when it is greater in the antigen urine tube

The second or lytic phase is determined by the buret test, both tubes being filtered and the filtrate stratified on an alkaline copper solution (10 per cent potassium hydroxide to which 25 cc per liter of 3 per cent copper sulphate is added), and read after incubation for twenty minutes

A positive buret reaction in the antigen urine filtrate and not in the control indicates tuberculosis

According to Piazza the reaction is positive when

- 1 The first phase is seen only in the urine antigen tube or is greater than in the neutral tube
- 2 The lytic phase occurs only in the first tube
- 3 Both phases occur in the same urine

BACTERIOLOGY Convenient Platinum Needle, Mudge, C S Am J Pub Health 20 195, 1930

Into a glass tube of suitable length, one end of which has been rounded in the flame, is inserted a copper wire so bent that a considerable pull is needed to dislodge it The platinum wire is fused to the end of the copper wire by heating the copper wire and thrusting the (red hot) platinum wire into its melted end

BLOOD CLOT CULTURE The Communion of Blood Clots for Cultures, Sellers, T F and Morris, J F Am J Pub Health 20 195, 1930

Satisfactory communion of the clot may be secured by placing it in the barrel of a sterile 10 cc Luer syringe, expelling the air, and pressing the clot through the nozzle of the syringe

BLOOD AMYLASE Value of Estimation in the Diagnosis of Pancreatic Disease Elman R Arnesson, N and Grahm, E A Arch Surg 19 943, 1929

A method is described based upon the viscosity of starch solution

Viscosimeter The instrument used is of the type originally designed by Ostwald. It is U shaped with a capillary tube and bulb inserted into one arm. The starch solution (5 c.c. of a 3 per cent solution) is introduced into one arm of the tube and, by suction on a rubber tube, is drawn above the upper mark into the other arm, whereupon the suction is released. As the level of the fluid passes this point the stop watch is started, and as the second mark just below the bulb is passed the watch is stopped. This time interval (in seconds) is taken as the measure of viscosity. Viscosimeters were selected which had an outflow time for water of from fifteen to twenty seconds or for the starch solution of from forty to fifty seconds. This short period enables one to make more rapid readings and gives values as accurate as those with tubes having a much longer outflow time. Several readings are made to check the constancy of the viscosity, and ordinarily 0.4 c.c. of the plasma to be tested is added. A few bubbles of air are blown through to insure mixing, and readings are made every few minutes until there is a 20 per cent reduction in the outflow time, that is, in the viscosity. The number of minutes required to reach this point is recorded. A graph may be plotted if desired with viscosity in seconds, and time in minutes as axes. It forms almost a logarithmic curve, and, as reported in a previous communication this time interval, in minutes, bears an inverse linear relation to the amount of amylase added, that is

the reaction follows Arrhenius rule $T = \frac{1}{Q}$ where T equals the time required to effect a given change and Q equals the concentration of enzyme used to effect this change. The addition of 0.4 c.c. of blood plasma to 5 c.c. of starch solution affects the initial viscosity so little that no correction is necessary for the zero point. This is due to the fact that the blood plasma has about the same viscosity as the starch preparation used. In the case of plasma with higher concentrations of amylase a smaller amount may be added than is 0.1 instead of 0.4 c.c.

A water bath with glass slides is used the back wall being frosted. A light behind it gives satisfactory illumination. The water is kept in circulation by air blown through it, and the temperature is maintained at $37.5^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ by means of a mercury thermostat and a small gas flame. The viscosimeters, as well as all pipettes are plugged with cotton to preclude the entrance of particles of saliva. All glassware is kept scrupulously clean, for any adhering particle may affect the passage of fluid through the capillary tube. As a routine, the following solutions are run through before being used each time: cleaning solution, tap water, alcohol and ether.

Starch Solution We found variations in the composition of various preparations of soluble starch so that for any series of observations it is necessary to obtain a good supply and to use the same preparation throughout. The batch that we are now using is made up as follows. Three grams are weighed out and added to 70 c.c. of cold distilled water and shaken until the suspension is homogeneous. It is then brought to boil over a free flame with constant shaking, in not less than three minutes, although a longer period does not alter the properties of the final solution. Thirty c.c. of Sorenson's fifteenth molar phosphate buffer are added (pH 6.8) and the flask is stoppered with a cotton plug and autoclaved for fifteen minutes at 10 pounds (4.5 kg.) pressure. Just before it is ready to use it is filtered through paper, and 5 c.c. portions are transferred to the viscosimeters. The solution, if kept sterile at 37°C may stand for from several hours to a day or two without altering its usefulness but if kept several days changes seem to occur. Blood is obtained by venipuncture, it is oxalated, and the clear plasma used. Standing in the ice box for from twelve to twenty-four hours does not alter its diastatic power but longer periods may do so.

Calculation of Amylase Units With 0.4 c.c. of normal oxalated plasma from the human adult a reduction of 20 per cent in the outflow time is effected in about thirty minutes, or with 0.2 c.c. in sixty minutes. One unit is arbitrarily taken as the amount of enzyme in 1 c.c. which will reduce the viscosity 20 per cent in one hour. The formula for determining amylase units (A.U.) on this basis is $A.U. = \frac{60}{TV}$, which is simply Arrhenius' rule with the factor of concentration added. In the foregoing formula, A.U. equals units

per cubic centimeter, T, time in minutes required to reduce the viscosity 20 per cent and V, volume of enzyme solution (plasma) used to effect this change

It was found convenient to have a solution of saliva or of pancreatic juice diluted so that 0.1 cc of it was as active as 0.4 cc of normal human serum. It keeps well in the ice box for several months or more, provided a crystal of thymol is added. In this way a standard control was available at all times without the necessity of obtaining normal plasma.

Uniform values (from 4.3 to 6.8 units) were found in a series of twenty-five unselected cases in which there was no suspicion of disease of the acini of the pancreas. The same "normal" range of values was found in eleven additional cases in which the pancreas was actually examined and found to be normal, seven of these by palpation at operation and four by microscopic section after autopsy.

Definite deviations from this "normal" range were found in twenty-one of twenty-three cases in which disease of the pancreas was found either at operation or at autopsy. In most instances a moderate or marked increase in the blood amylase was found (from 7.8 to 150 units), in some a definite decrease occurred (from 0.5 to 3.1 units). The significance of these observations has been pointed out.

The determination of blood amylase has been of undoubted clinical value in a number of cases both in excluding suspected disease of the pancreas and in adding conclusive confirmatory evidence when the clinical picture was indefinite or vague. Jaundice, per se, has no influence on the amylolytic power of human blood unless disease of the pancreas is also present.

NEGRI BODIES Rapid Method for, Petriagnani, G Bull Inst Sierat Milanese 7 557, 1928

The methods given are very useful for the rapid demonstration of Negri bodies and the mordant for staining bacteria. The author's mordant is as follows: Solution I ground potassium alum (cryst) 3 gm, lead acetate (cryst) 0.5 gm, glacial acetic acid 3 drops, distilled water 100 gm, dissolve on water bath and mix with Solution II tannic acid 7 gm, ferric chloride 2 gm, methyl alcohol (pure) 35 gm, distilled water 15 cc. Filter after two to three days, dilute with 50 or more volumes of methyl alcohol.

METHOD A (FOR SECTIONS)

Run through xylol and absolute alcohol. Treat five to ten seconds in dilute mordant, wash rapidly with absolute and 95 per cent ethyl alcohol, stain ten to twenty seconds with good eosin ("spiritus losch Grubler" specified, 0.5 gm in 100 cc 50 per cent ethyl alcohol), wash slowly with water, stain one minute with Mayer's hematoxylin (hematoxylin 1 gm, sodium iodate 0.2 gm, alum 50 gm, water 1000 cc), wash rapidly with water, stain with methylene blue (methylene blue Grubler 1 gm, water 1000 cc) until violet (if blue, it is overstained), dry with filter paper, wash and shake fifteen to twenty seconds with absolute alcohol containing 0.25 per cent N/2 NaOH, wash with 90 to 95 per cent ethyl alcohol until a general blue, two changes of absolute alcohol, xylol, mount in neutral balsam. Stains nerve cells blue, nucleus dark blue, capillaries with nucleus and endothelium brilliant red, red globules red, Negri bodies eosin red.

METHOD B

After xylol and absolute alcohol treat a few seconds with mordant diluted in 100 to 200 volumes, wash with absolute alcohol, then 75 per cent alcohol, stain thirty seconds with eosin as above or acid fuchsin (0.5 gm acid fuchsin Grubler or Kahlbaum in 100 cc 50 per cent alcohol), wash in water, then in 95 per cent alcohol, two changes of absolute alcohol, xylol, balsam. Stains cellular bodies pink, Negri bodies brilliant red. A very useful method for a rapid diagnostic stain.

METHOD C

Treat with mordant (1 vol in 20 to 40 vols methyl alcohol) five to ten seconds, with acid fuchsin as in Method B, for one minute, wash in water, stain with indigo carmine (indigo carmine Kahlbaum 1 gm, water 500 cc) for five to twenty seconds, wash with water, then 95 per cent alcohol, two changes of absolute alcohol, xylol, balsam. By this

method the indigo carmine partially displaces the acid fuchsin a good stain for demonstrating the chlamydozoic structure of Negri bodies

TISSUE Rapid Method for Demonstration of Mucin Little R D Bull Assn Med Mus
12 120, 1929

The method has been tested on normal and pathologic tissues fixed in formalin or Zenker Helly

1 Transfer paraffin sections through xylol and alcohols to water Treat with iodine and with sodium thiosulphate if the fixative used contained mercury

2 Transfer sections to a 2 per cent aqu sol of toluidin blue (no information given as to source or dye content) for one minute

3 Wash in water dehydrate in pure acetone (alcohol does not preserve metachromasy as well) clear in xylol and mount in neutral balsam

Results Mucin, reddish violet cell nuclei and bacteria deep blue red cells yellow or greenish yellow, cytoplasm, fibrous tissue, bluish green, thyroid colloid very pale blue decalcified bone, light bluish green with pale violet Sharpey's fibers cartilage matrix, deep bluish violet, hyaline and amyloid, bluish green caseous matter pale blue green coagulated serum, pale greenish blue, muscle light blue, and most cell granules are blue violet

HEMATOCRIT A Handy Double Purpose Pratt O E and Swartout H O Arch Path
9 69, 1930

For materials, one needs only a $\frac{5}{8}$ by 5 inch heavy walled test tube rubber stoppers and a graduated tube of the kind used in Sahli hemoglobinometers The rubber stoppers are readily shaped with the aid of a cork borer and a pocket knife The one in the bottom of the test tube fits snugly enough to prevent its falling out when the tube is inverted The upper one is fitted water tight The flange on this stopper prevents it from forcing itself down into the tube during centrifugation The device does not need to be taken apart for cleaning, and there is but little trouble with breakage

Take blood by venipuncture oxalate it and after careful mixing pipette from 2 cc to 25 cc into the inner tube of each of a pair of these devices then centrifugate the preparation at high speed until there is no further noticeable diminution in the volume occupied by the cells The upper levels of both cells and plasma are easily read on the scales of the Sahli tubes, the percentage by volume represented by the cells being calculated from the averages of the values thus obtained The clear plasma is then pipetted off for use in the determination of its carbon dioxide combining power in the regular way

TISSUE Combined Nuclear and Differential Stain Erelmyer G J Bull Internat Assn
Med Mus 12 122, 1929

This is a combination of Delafield's hematoxylin and Mallory's connective tissue stain The hematoxylin intensifies the nuclei and is in turn converted over to a red color by the action of the acid in the connective tissue stain The method does not require specific fixation.

1 Stain in Delafield's hematoxylin for five minutes wash

2 Stain in 0.2 per cent aqueous solution of acid fuchsin for one minute, wash

3 Stain for two to three hours in

Anilin blue (water solution)	0.5 gm
Orange G	20 gm
Phosphomolybdic acid, 1 per cent aqu sol	1000 cc

4 Wash and pass rapidly through 35 per cent 70 per cent, and 95 per cent alcohols Dehydrate in absolute alcohol Clear in xylol and mount

TISSUES Rapid Paraffin Method, Ambrogi, L P Bull Internat Assn Med Mus 12 124, 1929

Especially recommended for tissues which get brittle in chloroform Not suitable for tissues containing much air (lung)

- 1 Fix thin slices (2 or 3 mm) in 10 per cent formalin 6 hours
- 2 Running water two hours
- 3 Acetone, 3 changes, thirty minutes each
- 4 Cedarwood oil till transparent
- 5 Paraffin, 3 changes, six hours each

TISSUES Staining of Tubercle Bacilli, Haythorn, A R J Tech Meth 12 130, 1929

The solutions used in fixing, embedding, dehydrating, and mounting paraffin sections remove something from tubercle bacilli that is necessary for maintaining their acid fast qualities Zenker's fluid is better in this respect than formalin, formalin fixation should be used only when frozen sections are to be stained within forty eight to seventy two hours For such frozen sections and for material fixed in Zenker's fluid (containing 5 per cent acetic acid) using 5 to 10 times as much fluid as there is tissue to be fixed (followed by the usual steps of dehydrating, embedding, and sectioning), proceed as follows

Stain lightly (two to five minutes) in hematoxylin, decolorize in acid alcohol if over stained, place in tap water until blue Stain in Ziehl's carbol fuchsin one hour in paraffin oven at 55° C, wash in tepid water, then in ice water (8 to 10° C) decolorize in 10 per cent sulphuric acid, cooled by standing several minutes in ice water, until sections are pale violet, wash in ice water and repeat application of acid if too much red color returns, wash in ice water and stand in tap water until blue, remove sections one at a time, blot, wash rapidly with 95 per cent alcohol from a dropping bottle Flood with orange G, dissolved in absolute alcohol, from a drop bottle, until section is pale orange wash with absolute alcohol, blot and flood with xylol, blot and mount in balsam

TISSUE Stain for B Leptae and Myelin Sherth, Campbell, H Bull Internat Assn Med Mus 12 129, 1929

Fix tissue in acetic Zenker Wash, place for six to twenty four hours in equal parts 80 per cent alcohol and Lugol's solution Place in 80 per cent alcohol twelve to twenty four hours, 95 per cent alcohol two to six hours, absolute alcohol two changes, six to twenty four hours, absolute alcohol and xylol (equal parts) one half hour, xylol one half hour, paraffin two changes one hour After sectioning and securing to slide, place in xylol to remove paraffin, stain one half hour in Kinyoun's carbol fuchsin (basic fuchsin 4 gm, phenol crystals 8 gm, 95 per cent alcohol 20 cc, distilled water 100 cc) Rinse in water, acid alcohol (0.5 per cent HCl in 35 per cent alcohol), two changes, do not completely differentiate at this step Stain in Harris' hematoxylin without acetic acid two minutes or less Differentiate again in the acid alcohol, rinse in water, place in 1 per cent ammonia water, rinse in water Counterstain in 1 per cent aqueous orange G Dehydrate quickly in two changes of acetone, xylol, mount in xylol damar or xylol balsam

(No information given as to source of dye content of stains used)

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building,
Richmond, Va

*Diseases of the Blood*¹

THIS is one of the Harper Series of Medical Monographs intended to present to the practitioner in a single volume a survey of all the recent advances in a single subject

Knowledge of diseases of the blood has undergone considerable revision in past years and the subject, as presented by Clough, is brought up to date. The first seventy pages are concerned with a clearly put discussion of the blood cells their origins and functions and the more useful and common laboratory studies concerning them.

The diseases of the blood are then systematically discussed in each the etiology, symptoms, course, pathology, differential diagnosis, and treatment being covered. There is also a very practical chapter on blood transfusion short but well worth reading. The final chapter covers all that the practitioner need know concerning methods for the examination of the blood.

The practitioner will make no mistake in the purchase of this little volume.

Blood Grouping[†]

THE practical importance of blood grouping in the general field of medicine as well as its recent invasion of the field of legal medicine has given rise to a voluminous literature on the subject much of which has been relatively inaccessible.

This volume presenting as it does a comprehensive analytical survey of this complicated subject is, therefore, most timely. That it is authoritative is assumed by the fact that the author has contributed to its literature by extensive experimental observations that it is needed is evidenced by the perceptible degree of clinical confusion which exists concerning the entire subject.

Every phase of the subject is thoroughly discussed. The chapters on the medicolegal applications of blood grouping are exceedingly valuable and there is also four very useful chapters concerning transfusion.

This book will be a valuable addition to the reference library of the pathologist, the laboratory worker, the clinician, and in fact to all who are interested in the ramifications of medicine in general.

There is only one valid criticism. Despite the theoretical advantages of the new Landsteiner classifications and the efforts to introduce its general use as supplanting those of Moss and Jansky it has not and, in all probability will not be adopted. As it is used throughout the book, it is to be regretted that either the Moss or Jansky are not also given in parenthesis to facilitate the reader's orientation and perhaps to familiarize him when necessary with the Landsteiner terminology.

¹Diseases of the Blood. By P. W. Clough, M.D. Associate in Clinical Medicine, Johns Hopkins University. Cloth 310 pages. 2 colored plates. Harper and Bros. New York, N. Y.

[†]Blood Grouping in Relation to Clinical and Legal Medicine. By L. H. Snyder, Associate Professor of Zoology, North Carolina State College. Cloth 153 pages. 1 colored plate. 28 figures. Williams and Wilkins Co. Baltimore, Md.

NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

Coronary Thrombosis[†]

THIS volume presents an exceedingly interesting and valuable review of a subject, a clear clinical concept of which has only been achieved within comparatively recent years. It is only recent studies which have emphasized that coronary occlusion does not necessarily predicate immediate death, that its occurrence is compatible with a fair degree of health, and that it can be clinically recognized and distinguished from angina pectoris and status anginosus.

These facts are well brought out by Dr. Levin's study based upon 115 cases the histories of which form the final section of the book.

The etiology, symptoms and signs, differential diagnosis, prognosis, and treatment are all discussed in a clear, scholarly fashion and present a contribution of great value to the clinician. The volume is a worthy addition to the Medicine Monograph series and deserves wide circulation.

Clinical Medicine[‡]

THIS is a very practical book. So innumerable have been the advances in modern medicine that it is impossible to more than suggest many of them to the student confronted with the present-day medical curriculum. He must, therefore, rely after graduation largely upon his library which his medical education has taught him how to read, understand, and utilize.

The character of medical books has changed, however, and in place of the ponderous tomes and unalterable systems there are now the monographs and the loose-leafed compilations.

There is place, however, as Professor Bethel points out in his preface, for still another type of book, the practical volume suggesting the management of disease in the home where the vast resources of the hospital and clinic can neither be afforded nor easily called upon.

Such a volume is Bethel's *Clinical Medicine* which discusses from the standpoint of a wide and varied experience approximately one hundred of the more common conditions in the field of internal medicine.

The method of presentation is eminently practical and emphasizes the apt quotation cited: "It is not seeing rather than not knowing which gets us into trouble." Bethel emphasizes the necessity for *examining* the patient and for applying common sense to the problem at hand.

The chapters on overweight and underweight are typical of the manner of the book and depict the real management of patients and their troubles. This book should be welcomed by the physician at large.

Hypertension and Nephritis[‡]

BY THE publication of this book Dr. Fishberg has done an inestimable service to the practicing physician.

The introduction of the sphygmomanometer and the evolution of methods for the study of blood chemistry have greatly advanced the understanding of kidney function and its relation to health and disease in general. While this subject has been greatly clarified by modern studies it has not yet been entirely elucidated, while much has been added to its understanding, its complications and ramifications have also become clearer and better recognized and it is to a consideration of these that this book is directed.

It is of special significance to note that the title is not "Hypertension IN Nephritis" but "Hypertension AND Nephritis," for it presents a much needed and comprehensive considera-

*Coronary Thrombosis: Its Various Clinical Features. By S. A. Levine, Senior Associate in Medicine, Peter Bent Brigham Hospital. Cloth, 85 figures, 1 colored plate, 178 pages. Williams and Wilkins Co., Baltimore, Md.

†Clinical Medicine. By O. W. Bethel, Professor of Clinical Medicine, Tulane School of Medicine, etc. Cloth, 700 pages. W. B. Saunders Co., Philadelphia.

‡Hypertension and Nephritis. By A. M. Fishberg, M.D., Attending Physician, Mt. Sinai and Montefiore Hospitals, New York. Cloth, 506 pages, 33 engravings, 1 colored plate. Lea and Febiger, Philadelphia.

tion of that large group of cases of what, for lack as yet of a better term, are known as "essential hypertension"

The entire volume, while comprehensive in its survey of the literature of a complicated subject which has undergone intensive investigation, is written from a clinical standpoint and primarily for the practitioner

The modern resources of the laboratory which have added greatly to the subject are well covered but, very wisely, the clinical avenues of approach are emphasized and cited in detail for as clinical pathologists are among the loudest in declaiming, clinical studies should *precede* and *suggest* the appropriate laboratory procedures which are essentially additional methods for determining the reaction of the organism to stimuli the nature and degree of which must be suggested not only by the laboratory report but particularly by its interpretation

Moreover as many patients cannot afford extensive laboratory investigations for which many physicians do not have the necessary equipment technical skill, or expert assistance, the simpler methods of definite value are well covered

The first nineteen chapters are given to the discussion of renal dysfunction, their mechanism recognition, and treatment and are followed by four excellent chapters upon the present conception of essential hypertension.

The final chapter treats of renal and hypertensive disease in pregnancy. The treatment of eclampsia given is that of Stander and Williams, the work of Titus and others not being cited

Both the publishers and the author are to be congratulated on a useful and valuable addition to the literature of the subject

The book can be recommended as well worth perusal and study

Clinical Methods¹

IT IS a logical assumption that a book does not reach its ninth edition unless it is of value to its readers, and perusal of this extensively revised edition of a well known manual proves the assumption to be well founded

As indicated in the preface the purpose of the book is to suggest the available means whereby a particular clinical problem may be handled or in other words, to answer the question "How shall I investigate this case?" The volume not only shows what should be done but also how to do it

It may be asserted with confidence that any man who studies his cases in accordance with the methods described will not only be practicing medicine as it should be practiced but will inevitably add greatly to his own and the knowledge of others

The volume should be read and reread by every practitioner

An Introduction to Biophysics†

JUST as biochemistry deals with the application of chemical knowledge to living substances biophysics deals with the application of physics and physical chemistry to the study of living substance. Burns rather limits this general subject in his volume to the application of physical and physical chemical methods to the study of human physiology. It is primarily a textbook and unless one is recently well grounded in his physics and has rather more than the average acquaintance with mathematics it will be hard digging. We rather envy the present day medical student who will have this served to him in as appetizing manner as possible while we older folks must necessarily make rather a grim business of digesting it

Clinical Methods. A Guide to the Practical Study of Medicine. By R. Hutchison, M.D. Physician to the London Hospital and Donald Hunter, M.D. Assistant Physician to London Hospital. Cloth. 684 pages. 1/2 figures. 18 in colors. Ninth edition. P. B. Hoeber, New York.

†*An Introduction to Biophysics.* By David Burns, M.A., D.Sc. Professor of Physiology in the University of Durham. Late Grieve Lecturer on Physiological Chemistry in the University of Glasgow. With a foreword by Prof. D. Noel Paton, M.D., LL.D., F.R.S., etc. Second Edition. With 116 illustrations. cloth. pages 680. The Macmillan Company, New York, 1929.

The earlier physiologist had to be content with recording phenomena rather than with explaining them. Their interest was more in "what happened" than in "why it happened." The second question was always uppermost in their minds but they did not have the back ground of information with which to explain. From a mechanistic viewpoint this volume answers many of the previously unanswered questions of physiology. The physiologist of today has come to depend more and more upon physics and chemistry to explain the phenomena which he observes. Dr. Burns demonstrates in this volume the part that physics plays in their explanation.

The first section is practically pure physics. The second deals with the application of the physical facts described, to the mechanics of protoplasm and cells. The third section carries on the development of the subject to cell communities or aggregations, the fourth to systems of the body, while the fifth describes the biophysics or physical physiology of the organism as a whole.

Qualitative and Volumetric Analysis for Medical Students

A SMALL laboratory manual for qualitative and volumetric analysis developed for the use of medical students especially in preparation for their final examinations. It is the type of volume which is especially applicable to the community for whose special needs it was developed and will undoubtedly find a wider use in England than in this country.

Diseases of the Blood†

A SMALL reference volume on the blood and its diseases developed primarily for the clinician and free from the exposition of pet theories. Most of the facts presented can be found scattered through larger treatises and systems but this volume possesses the advantage of condensation and systematization.

The illustrations are excellent.

Laboratory Manual of the Massachusetts General Hospital‡

A MANUAL of the routine and special procedures in use at the M. G. H. Excellent for quick laboratory reference work. Most of the "occasional" examinations are in it and are described in sufficient detail. Among these we might pick out merely as illustrations special examination for hookworm ova, reticulocyte counts, technique of measuring the diameter of red cells, fragility test for red cells, blood grouping, technique for orthotector specimens, blood cultures, methods of inserting the duodenal tube, method of chest tap, pericardial tap, abdominal tap, external puncture, pneumococcus typing, Van den Bergh test, liver function tests, vital capacity, etc.

Some therapeutic technique is also included such as intraperitoneal infusion, arsenphenamine injection, Swift-Ellis treatment, intracysternal treatment, intravenous administration of orthoiodoxy benzoic acid, intravenous administration of glucose, transfusion, and the various prophylactic vaccinations and inoculations.

This volume should be of great value not only to the laboratory man but to the clinician.

*Qualitative and Volumetric Analysis for Medical Students. By H. Lambourne. M.A. M.Sc. F.I.C. Head of the Chemistry Department. The Polytechnic, Regent Street, W. 1 and J. A. Mitchell. M.Sc. Lecturer in the Chemistry Department. The Polytechnic, Regent Street, W. 1. Cloth. 64 pages. Humphrey Milford, Oxford University Press, New York, 1923.

†Diseases of the Blood. By A. Piney. M.D. M.R.C.P. Research Pathologist, Cancer Hospital, London. With 20 illustrations, six in color. Cloth. pages 195. P. Blakiston's Son & Co., Philadelphia, 1923.

‡Laboratory Manual of the Massachusetts General Hospital. By Roy R. Wheeler. M.D. and F. T. Hunter. M.D. Second edition, enlarged and thoroughly revised. pages 101. cloth. Lea & Febiger, Philadelphia, 1923.

*An Introduction to Medical Protozoology*¹

THE most complete volume on the protozoan infections of man which has appeared in the last few years, written by a man eminently fitted for the work by virtue of his position as professor of protozoology in the Calcutta School of Tropical Medicine. There is incorporated a discussion of spirochetes together with several chapters on laboratory methods.

Green's Manual of Pathology and Morbid Anatomy†

ONE might say that the fourteenth edition of any book requires no introduction but Green's Pathology edited by Pincus is to a certain extent an exception since it is a British book and has found its reading public primarily in the British Empire.

This is essentially a textbook of pathology for medical students and reference manual for the practitioner, the major interest of which is morphologic pathology. The illustrations are excellent and are abundant. There is only one chapter which appears to have been unnecessarily abbreviated that on Malformations.

The work can well be recommended as an authoritative reference volume on gross and microscopic pathology.

The Biochemistry of the Amino Acids‡

THIS review sponsored by the American Chemical Society brings the known facts on the amino acids up to date in one comprehensive volume. Usually a reviewer becomes enthused or otherwise with the body of a book. There is plenty to be enthused over in the main body of this contribution since the subject matter is remarkably well handled. This time however the reviewer finds himself enthused especially over the preface. It is one of the best written prefaces we have seen in a long time. Unfortunately it does not lend itself well to abstraction and should be read in the original. The reader who customarily starts in on Chapter I will miss much of the spirit of the review if he overlooks the preface.

The volume is not a mere compilation but is distinctly a critical review into which the authors have had no hesitancy in introducing their own opinions and criticisms.

The work deals successively with the physico-chemical properties of the amino acids, the determination and recognition of amino acids, digestion of protein in the intestinal tract, absorption, anabolism, catabolism and utilization of amino acids, special phases of amino acid metabolism, the dynamic effect of amino acids, endogenous catabolism and the nutritive value of protein.

The volume will be of reference value to biochemists, physiologists and clinicians.

Outline of Bacteriology§

A SHORT outline of general bacteriology developed on the basis of the author's lectures in oral hygiene at Columbia University. This volume is for the use of elementary students in bacteriology, especially those who anticipate only a bird's-eye view of the subject. It would be appropriate for nurses, students of social hygiene and the like but is not sufficiently detailed for students of medicine.

¹An Introduction to Medical Protozoology with chapters on the Spirochaetes and on Laboratory Methods. By Robert Knowles, B.A. (Cantab.), M.R.C.S., L.R.C.P., Lt. Col. Indian Medical Service, Fellow of the Asiatic Society of Bengal, Professor of Protozoology, Calcutta School of Tropical Medicine. Illustrated cloth, pages 85. Thacker Spink & Co. Calcutta. 1938.

†Green's Manual of Pathology and Morbid Anatomy. By A. Pincus, M.D., M.R.C.P. Research Pathologist, Cancer Hospital, London. Late Director of the Institute of Pathology, Charing Cross Hospital, London. Some time Lecturer in Pathological Histology in the University of Birmingham. Fourteenth Edition. Revised and enlarged cloth, illustrated, pages 600. Lea & Febiger, Philadelphia and New York. 1938.

‡The Biochemistry of the Amino Acids. By H. H. Mitchell, Professor of Animal Nutrition, College of Agriculture, University of Illinois, and T. S. Hamilton, Associate in Animal Nutrition, College of Agriculture, University of Illinois. American Chemical Society Monograph Series. Cloth, pages 619. Book Department, The Chemical Catalog Company, Inc., New York, 1939.

§Outline of Bacteriology. By Henry A. Bartels, B.S., D.D.S., Lecturer on Bacteriology, School of Oral Hygiene, Columbia University, Instructor, Dept. of Oral Pathology, School of Dental and Oral Surgery, Columbia University. Cloth, pages 123. William Albert Broder, Publisher, New York. 1939.

*The Principles of Clinical Pathology in Practice**

THIS is not a book on laboratory methods. Indeed it contains practically nothing of laboratory methods. It is offered to the physician primarily as a reference manual to aid him in interpreting reports which come to him from the laboratory. The general procedure is to give a brief general description of the individual disease, tabulate the abnormal findings in this disease which may be reported from the laboratory, and then discuss in more detail each of these findings as they apply to this disease.

One using this manual would therefore have to make a tentative diagnosis, read over the description of the general features of this tentative disease, see what examinations should be made, and, after having received the reports on these examinations from the laboratory, read the final section to see how the actual reports correspond with what should be anticipated for that disease.

This arrangement is not so different from that found in the average textbook of medicine. The chief difference lies in the abbreviation of general considerations under each disease, and the enlargement on the discussion of the laboratory findings. The grouping of the diseases is original but probably in actual practice does not facilitate particularly the placing of an unknown symptom complex in its proper bracket.

A final chapter on methods of collecting specimens and transferring them to the laboratory should be especially helpful.

The Doctor in Court†

A MOST enjoyable series of court anecdotes most of which were written in the personal experience of the author. While it is written in humorous vein, the object is deeper, to bring before the medical profession and the public the great shortcomings of present day legal procedure especially as it applies to medicolegal work and expert testimony. Its perusal by no means increases one's respect for the processes of law.

There are many excellent bits of advice for any physician who may be called upon the witness stand. This volume makes a delightful evening's reading.

The Blood Plasma in Health and Disease‡

THE scope of this volume does not turn out to be as broad as would be indicated by the title. It is, essentially, an exhaustive treatise on the clotting of blood and the biochemistry of those blood constituents which enter into the process of thrombosis. It should serve well as a reference manual for those who are interested in an experimental way in this phase of physiology and biochemistry.

*The Principles of Clinical Pathology in Practice. A guide to the interpretations of laboratory investigations for the use of those engaged in the practice of medicine. By Geoffrey Bourne M.D. (Lond.) M.R.C.P. Casualty Physician, Demonstrator of Practical Medicine and Chief Assistant to the Cardiographic Department, St. Bartholomew's Hospital, Senior Physician, East London Hospital for Children, Shadwell E. and Kenneth Stone M.D. (Oxon) M.R.C.P. Late senior Demonstrator of Pathology, St. Bartholomew's Hospital. Cloth, pages 392. Humphrey Milford, Oxford University Press, American Branch, New York, 1929.

†The Doctor in Court. By Edward Huntington Williams M.D. Cloth, pages 239. The Williams and Wilkins Company, Baltimore, 1929.

‡Monographs of Medical and Surgical Science. Edited by Professor R. J. S. McDowall D.Sc., M.B., F.R.C.P. (Edin.), University of London, King's College. The Blood Plasma in Health and Disease. By J. W. Pickering D.Sc. (Lond.), Lecturer on Hematology, University of London, King's College. Cloth, pages 247. The Macmillan Company, New York, 1928.

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EDITORIALS

The Serum Therapy of Meningococcic Meningitis

OVER a quarter of a century has passed since the introduction by Flexner in 1909 of a specific serum for the treatment of meningococcic meningitis, and until very recently the serum treatment of this disease has been clinically satisfactory, the death rate being appreciably reduced.

Within recent times, however, occasional cases have been encountered in which the use of antimeningococcic serum was without avail, and a recent report by Wright, De Sanctis, and Sheplar¹ records observations of particular interest and significance.

With the entrance of the first case of meningococcic meningitis into their wards in 1928, these observers were startled and chagrined to find that the hitherto standard and effective serum treatment was valueless. The immediate natural and logical assumption was that the organism in question was a serum resistant strain, and, before an effective serum could be found, ten deaths were recorded.

So far, an entirely satisfactory method of standardization for antimeningococcic serum is yet to be found, and dependence has been placed upon the presence in the serum of agglutinins for the meningococcus strain in question. The observations recorded emphasize that unqualified dependence cannot be placed upon the agglutinin titer as a reliable measure of therapeutic efficiency.

Of seven different makes of serum tested by Wright, De Sanctis, and Sheplar one was particularly high in agglutinin content yet clinically ineffective, and as similar findings were encountered with other serums, the authors call attention to the fact that the agglutination test is unreliable as a method of selecting a potent serum against a given strain of meningococci.

The fact that one serum may be useless and another strikingly effective again brings up the still unsolved problem of how best to prepare antimeningococcic serum.

The practical difficulties, and the enforced delay, attendant upon the use of monovalent serum has led to the universal use of polyvalent serum. The question arises, then, as to how many strains shall be used in its preparation.

It is apparent that to be of therapeutic value in a given case a serum must be specific for the meningococcus strain involved, and in the endeavor to encompass this an impression has arisen that the extent of effective polyvalency is related to the number of strains used in the preparation of the serum. Clinically, however, the report under discussion does not confirm this impression, thus corroborating the observation of Wadsworth² that the presence of a large number of strains decreased the potency of the serum against any single strain.

That this is undoubtedly true, the clinical experience of Wright, De Sanctis, and Sheplar amply demonstrates.

It is of some interest, therefore, to inquire as to the number of strains used in the commercial preparation of antimeningococcic serum and such an inquiry of three prominent firms elicited that one used the four Gordon strains, one used the four Gordon strains to which were added "from time to time strains from epidemic areas" in numbers not stated, while the third, in addition to the four Gordon strains used thirteen additional strains isolated from cases in various parts of the country.

In view of the absence of any relation between agglutinin titer and therapeutic efficiency, the work of Schwartzman³ is of interest.

As a result of investigations upon the local skin reactivity to culture filtrates of the typhoid bacillus this observer reported that the two factors involved, the "skin preparatory factor" and the "reacting factor" could be consistently titrated, that both types of factors can be specifically neutralized by immune serums, and that the neutralizing potency of antityphoid serums may thus be quantitatively measured.

This work has now been applied to the standardization of antimeningococcic serum with highly encouraging results (applicable so far, however, only to Type I meningococcus) and with indications that its application may be still further broadened.

The present status of the matter can be no better outlined than by repeating the conclusions of Wright, De Sanctis, and Shepler

1 The agglutination test does not give uniform results, and is unreliable as a guide for determining the value of a certain serum against a specific strain of meningococcus

2 As other methods such as the opsonic index and the complement fixation test have not been of proved value in this determination, the therapeutic test appears to be the only reliable method of determining the curative value of a serum against a specific organism

3 This test may result in fatality in the treatment in an individual case, yet it is invaluable as in our experience, in the treatment in a series of cases

4 When a patient with meningococcus meningitis fails to respond to treatment one cannot justifiably conclude that the strain of organism encountered is resistant to serum therapy but only that the serum used is not specific for that organism. It is necessary then to seek an effective serum

5 There appears to be a loss in specificity against a certain strain of meningococcus when too large a number of strains have been used in the production of a polyvalent serum

6 A single intraspinal injection of 20 cc of an effective antimeningococcus serum in each twenty four hours was therapeutically adequate. Potency of the serum rather than frequency of injections proved to be the essential factor for successful treatment

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—R A K

Erratum

In the article by Bryant, 'A Potassium Ferrieyanide Method for the Determination of Reducing Substances Present in Blood' August 1929 issue page 1082, the word 'ferrieyanide' in the first line page 1084, should be iodide "

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News and Notes

To the Members of the American Society of Clinical Pathologists

The Research Committee calls attention to the fact that all manuscripts in competition for the Ward Burdick Annual Award must be in the hands of the Chairman by April 30, 1930. It is hoped that there will be a large number of papers submitted.

In an endeavor to present the experiences of the entire membership on the subject of "Agranulocytosis," a questionnaire will be sent out within a few weeks. Meanwhile we would like all the members to assemble their cases, including those fitting the original description of Schultz's Agranulocytic Angina, and other cases showing marked evidences of specific injury or aplasia of the granulocytic elements of the blood or bone marrow. Cases following arsphenamine injections are particularly desired.

Reports will be collected at the same time on any experiences of the Society in regard to inoculation of chickens with Hodgkin's disease material to determine whether or not L'Esperance's results, namely, production of avian tuberculosis, can be corroborated.

Finally, an attempt will be made also to sum up the number of cases of undulant fever discovered since last year.

Research Committee,

A G Foord, M D, Chairman,
322 Jewett Avenue, Buffalo, New York

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CLINICAL AND EXPERIMENTAL

A STUDY OF THE RELATION OF THE BLOOD SUGAR IN PLASMA TO THAT IN THE CORPUSCLES IN NORMAL AND DIABETIC INDIVIDUALS*

By HENRY J JOHN MD CLEVELAND, OHIO

A COMPARISON of the sugar content of the blood plasma with the sugar content of the corpuscles shows, as a rule a variation in both directions, in other words, the level of the corpuscular sugar may be slightly higher or slightly lower than that of the plasma. In 1923 I undertook a study¹ of this relationship with the material which was then available and the present work is merely a continuation of this former study carried out with more extensive material. The data given in this paper have been collected from the study of the blood in 193 glucose tolerance tests 103 of which were made in diabetic, and 90 in nondiabetic patients.

The tests were performed in a routine manner and were always made while the patient was fasting. A specimen of blood was first taken after which the patient received 100 gm of anhydrous glucose dissolved in 250 to 300 cc of water to which the juice of one lemon had been added. This was made more palatable by being chilled. In the case of children under fifteen years of age, only 75 gm of glucose were used. Specimens of blood were again taken one half hour, one hour, two, three and four hours after the ingestion of the glucose.

The sugar content of the whole blood, of the corpuscles, and of the plasma was determined in the following manner. The oxalated blood was centrifuged at high speed for fifteen minutes, the plasma was drawn off and one cc of the corpuscles taken for the sugar determination. The corpuscles

From the Cleveland Clinic

were not washed Myers' modification of the Benedict method of blood-sugar estimation was used throughout these tests, the readings being made by means of the Kobei colorimeter In this study the plasma sugar was taken as a standard with which other estimations were compared, and the relative increase or decrease of the sugar was then calculated

The plasma contains a certain amount of sugar in solution, and since the corpuscles are surrounded by the plasma, the question arises as to whether or not an equilibrium may be reached On the other hand, the corpuscles are living bodies and consume sugar, just as do the tissues, while the plasma is simply a vehicle to supply energy to the corpuscles in the form of sugar Consequently, even though an equilibrium might be reached, one would perhaps expect to find the sugar content of the corpuscles lower than that of the plasma, especially if the plasma sugar were higher than normal If this were the only factor, then the sugar content of the corpuscles should not rise above a certain level, in other words, it should be constant under all conditions That this is not the case can easily be demonstrated, simply by a comparison of the sugar content of the corpuscles in the blood of a normal individual with that in the blood of a patient with marked hyperglycemia In the latter case the sugar content of the corpuscles will be high, and one is convinced that the factor of osmosis plays a rôle here, since the red corpuscle cell is quite different in this respect from a muscle cell

The red corpuscle, which is completely surrounded by the sugar-carrying plasma, absorbs by osmosis enough sugar to supply its own energy plus the amount necessary to establish an equilibrium between the two sides of the membrane We are dealing here with several inconstant variables (1) Changes in the sugar content of the plasma, which is constantly giving off sugar to the tissues (2) The intake of sugar into the blood stream from the stores in the liver, which is also not a strictly constant phenomenon (3) The utilization by the corpuscles of the sugar within their walls and the consequent decrease in their store of sugar (4) Osmosis of sugar through the corpuscular membrane, first, to replace that which has been used, and second, to equalize the pressure on the two sides of the membrane

A comparison of the changes in the sugar content of the corpuscles and of the plasma in Table I (nondiabetics) shows that in general the sugar content of the corpuscles is lower than that of the plasma, and that as the sugar level of the plasma rises, following the ingestion of glucose, the sugar level of the corpuscles does not keep pace with it As the sugar starts to decrease in the plasma, the corpuscles release their sugar less rapidly and we find a higher sugar level in the corpuscles This confirms the observations of Gradwohl and Blaivas² An examination of Table II (diabetics) shows conditions somewhat different from those in nondiabetic subjects Thus, in diabetic individuals, the level of the corpuscular sugar is lower than that of the plasma sugar and only rarely does one find at the end of the test that the sugar content of the corpuscles is higher than that of the plasma From a comparison of the two tables one gets the impression that in the presence of diabetes the corpuscles do not take in the sugar as rapidly as is normal, and that con-

sequently their sugar content stays for the most part below that of the plasma. Perhaps the lack of insulin may have something to do with this storing of sugar within the corpuscular wall, just as it does in the case of tissue cells. The corpuscles of diabetic individuals, therefore, differ from the corpuscles of normal individuals by their lessened permeability or capacity to take in and to hold sugar.

A study of Tables I and II shows that in 246 estimations of the blood sugar made in nondiabetic individuals, and in 44 in diabetic individuals there was an increase in the corpuscular sugar as compared to the plasma sugar the average increase in the nondiabetic cases being 17.12 per cent and in the diabetic cases 9.95 per cent. On the other hand, in 236 estimations in nondiabetic individuals and in 511 in diabetic individuals, there was a decrease in the corpuscular sugar as compared with the plasma sugar the average decrease in the nondiabetic cases being 12.20 per cent and in the diabetic cases 15.82 per cent. (These comparative data are given in Table III.) We may say therefore that among the nondiabetic individuals the ratio of the average increase in the corpuscular sugar over the plasma sugar to the average decrease was as 17.12 to 12.20, while the corresponding ratio among the diabetics was as 9.95 to 15.82. Thus among nondiabetic individuals the level of the corpuscular sugar was higher than that of the plasma sugar while among the diabetic subjects the level of the plasma sugar was higher in two thirds of the estimations. These figures emphasize the fact that in the presence of diabetes the blood corpuscles are on the whole, much less permeable to sugar than they are normally.

As for cases of severe diabetes, that is those in which the blood sugar at the beginning of the glucose tolerance test was 250 or above, it is of interest to see whether or not the ratios remain the same. An analysis of 85 blood sugar examinations in individuals with severe diabetes gave the following results:

Total number of examinations	85
Total decrease	1,427
Average decrease in corpuscular sugar as compared with plasma sugar	16.79 per cent
Increase in corpuscular sugar as compared with plasma sugar	0

The average decrease in the corpuscular sugar as compared with the plasma sugar, therefore, was practically the same as that observed in the total number of observations.

Another attempt to measure the permeability of the red corpuscles directly was made by the following method. The corpuscles were suspended in a solution of 1,000 mg. per 100 c.c. of glucose in N/10 salt solution for two hours. The corpuscles of the nondiabetic individuals were then compared with those of the diabetics (Table IV). It was found that the actual percentage of increase in corpuscular sugar among the nondiabetic individuals

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[illegible]

was 321 per cent, while among the diabetic subjects it was only 185 per cent. This test, therefore, confirmed our previous observation that in diabetes the corpuscles are less permeable to sugar than they are normally. Whether in diabetes the corpuscles have more nearly reached their saturation limit cannot be stated, but one would hardly suspect that to be the case, as a very high sugar content of the corpuscles may be observed in cases of diabetes, and this would indicate a tremendous capacity for taking in sugar.

TABLE III

A COMPARISON OF THE CORPUSCULAR SUGAR WITH THE PLASMA SUGAR IN NONDIABETIC AND DIABETIC CASES

	NONDIABETIC	DIABETIC
Total number of examinations	497	572
Total per centile increase	4212	438
Total number of examinations	246	44
Average per centile increase	17.12	9.95
Total per centile decrease	2879	8084
Total number of examinations	236	511
Average per centile decrease	12.20	15.82
Number of cases in which corpuscle sugar equalled plasma sugar	20	17

TABLE IV

PERMEABILITY OF RED BLOOD CELLS TO SUGAR
CORPUSCLES EXPOSED TO 1000 MG OF GLUCOSE PER 100 C.C. OF N/10 CaCl SOLUTION

	NONDIABETIC	DIABETIC
Total number of cases	19	23
Total per centile increase of corpuscular sugar	6100	4261
Average per centile increase of corpuscular sugar	321	185

That corpuscles should not be centrifuged and then washed by normal saline solution before their sugar content is estimated, as has been done by some authors, can be clearly seen from Table V. When the corpuscles are washed part of the sugar is washed out of them, for osmosis does not cease, just as when corpuscles are suspended in a hypertonic sugar solution, sugar is introduced into them. Therefore the washing of corpuscles before their sugar content is estimated invalidates the results of the test, and data compiled from such estimations must be disregarded.

TABLE V

THE LOSS OF SUGAR FROM THE CORPUSCLES AS THE RESULT OF WASHING THEM WITH NORMAL SALINE SOLUTION

		PER CENT	PER CENT LOSS
Case I	Corp. sugar before washing	204	100
	Corp. sugar after 15 minutes	94	46
	Corp. sugar after 30 minutes	23	11
Case II	Corp. sugar before washing	170	100
	Corp. sugar after 15 minutes	78	46
	Corp. sugar after 30 minutes	38	22

CONCLUSIONS

1 The sugar content of the red blood corpuscles in the diabetic individual is strikingly lower than the sugar content of the surrounding plasma

2 As the amount of the sugar in the blood of the diabetic individual increases, the increase occurs first in the plasma and does not appear until later in the red corpuscles. As the level of the plasma sugar falls the corpuscular sugar as a rule keeps pace with it. Thus one rarely finds a higher sugar level in the corpuscles than in the plasma at the end of a glucose tolerance test, when the blood sugar curve is descending.

3 This relationship is not altered in severe cases of diabetes that is, in cases in which the blood sugar is very high.

4 In general in the nondiabetic individual the corpuscles have a lower sugar content than the plasma, but this is not so marked as in the diabetic subject.

5 In the nondiabetic subject, when the sugar in the plasma increases the corpuscular sugar rises more than it does in the case of a diabetic but when the sugar curve is descending the corpuscles do not release their sugar so freely, and thus as a general rule, one finds a higher level in the corpuscular sugar than in the plasma sugar at the end of the curve.

6 A study of 1069 blood sugar estimations shows that in nondiabetic individuals the ratio of the average increase of the sugar in the corpuscles, as compared with that in the plasma, to the average decrease was 17.12 to 12.20, while in diabetic individuals the corresponding ratio was 9.95 to 15.82.

7 By suspending red corpuscles in 1,000 mg per 100 cc of glucose in a N/10 NaCl solution, the average increase of the corpuscular sugar after two hours was found to be 321 per cent in corpuscles from nondiabetic individuals, and 185 per cent in those from diabetic individuals.

8 The washing of corpuscles in normal saline solution before their sugar content is estimated is contraindicated as the sugar is washed out of the corpuscles and the estimations are therefore of no value.

9 The present study would suggest that in diabetes the corpuscles have a lesser capacity to take in and to hold sugar than normally. Whether it is the decreased amount of insulin present or some other condition which is the dominant factor, future studies should disclose.

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EXPERIMENTAL STUDIES IN DEPROTEINIZING BLOOD SERUM

PRELIMINARY REPORT

BY GEORGE TURNER, M D , EL PASO, TEXAS

THE chemical method of precipitating protein from solution by the use of the salts of heavy metals, and also by the use of "alkaloidal reagents," is a familiar study in biochemistry

This experimental method employs heavy metals, not as a salt, but in their pure state, reacting through the application of an electric current

For experimental purposes, a cell is prepared by fusing small gauge platinum wires through the wall of a large-size test tube. The fusing makes a "water tight" joint, and leaves one end of each wire protruding into the lumen of the tube. Upon the ends of the wires inside the tube are hung metal plates which constitute the electrodes. To the ends of the wires outside the tube are attached the lead wires from the generator. Other metals may produce the same physiochemical reaction, but the only one I have so far experimented with successfully has been pure gold.

When blood serum in its pure state or diluted with physiologic or hypertonic salt solution is placed in the cell, and a direct electric current applied, the protein (both primary and secondary) gradually accumulates upon the anode. If the serum is undiluted or slightly diluted, the protein will remain adherent to the gold anode. When the protein is all precipitated, the current is shut off, and the anode lifted from the cell with the protein adherent to it. If the serum is highly diluted and fresh, the protein will shed from the gold anode and settle to the bottom of the cell so that the protein-free solution can be pipetted off from above and between the electrodes. Platinum makes an excellent electrode, but will not cause the precipitation of protein when used as the anode, consequently the use of a platinum plate as the cathode and a gold plate as the anode makes a suitable cell.

Fig 1 is a photostat of one of the cells used. It is a glass tube, open at the top. The short plate in the picture is the platinum cathode and the larger plate is the gold anode. The amount of serum or serum solution placed in the cell should just cover the electrodes. The current may be furnished by any machine that will produce a direct current. The amount of current has been varied between 100 volts with 30 millamperes and 20,000 volts with 8 millamperes, with the same deproteinizing action.

The remaining protein-free solution is slightly alkaline. The degree of alkalinity will depend upon the amount of protein that has been precipitated and the hypertonicity of the solution. The alkalinity of the electrolyte can be held at the original P_H of the antitoxin by measuring the platinum cathode in a dialyzer and keeping the plate bathed in neutral physiologic salt solution. If the serum is undiluted or slightly diluted, it is best to make the solution hypertonic to insure and hasten the complete precipitation of the protein.

To determine if all the protein has been precipitated a small portion of the solution can be pipetted from between the electrodes and tested. If the serum is highly diluted the solution may be boiled as pipetted from the cell and if the protein is removed the solution remains perfectly clear after boiling. If the serum is concentrated, as in the case of diphtheria antitoxin, there will remain in solution so much antitoxic substance which is itself precipitated by heat, that the boiling of the solution as pipetted from the cell will not signify the complete disappearance of the protein. In this case, a preliminary step is necessary before boiling. As the solution is all alone after the application of the current the antitoxic element of the serum will remain in alkaline solution but is immediately precipitated when the solution is made

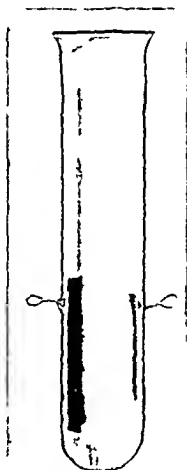


Fig 1—Cell for electrically precipitating serum protein

very slightly acid. Consequently, it is necessary to render the portion removed for protein test very slightly acid and filter before boiling.

The purpose of this method of deproteinizing serum is to precipitate the protein substance and remove it from the antibodies or antitoxic elements. This leaves these elements in a slightly alkaline salt solution. The antitoxic elements keep better, act quicker and with greater precision after the protein is removed. This improves the application of the complement fixation test, as well as antitoxins for therapeutic use.

Hemolytic serum or amboceptor, as used in the Wassermann test is simply the blood serum of a rabbit that has been immunized to the red blood cells of, usually, the human being or sheep. I have deproteinized antiserum, after which it acts more precisely than before deproteinization. A portion of the deproteinized serum, diluted to titer, was left at room temperature

for twenty days At the end of that time, none of its hemolytic property had been lost

The syphilitic antibodies remain after deproteinizing the serum of a syphilitic patient A positive Wassermann is obtained just the same after removing the protein as before The only difference is that the test on the deproteinized serum behaves more precisely, and positive results are obtained with less serum If the patient does not have syphilis, the test is clearly negative

Gonococcus fixation tests are clearer

It seems that in tuberculosis the elements separated are both toxin and antitoxin If the patient has active tuberculosis with temperature there are no antibodies present, but tuberculin is present If the patient does not have active tuberculosis with increased temperature there is a certain measure of tuberculous antibodies present This is indicated by the fact that tuberculin will increase the rate of hemolysis in the hemolytic phase of the complement-fixation test This is shown by running complement-fixation tests upon the deproteinized serum of tuberculous and nontuberculous patients, using a carefully standardized old tuberculin as antigen (The antigen should also be deproteinized after dilution) I have made twenty of these tests from patients at Dr R B Homan's Sanatorium, who were selected by him The sera were each given a number and sent to my laboratory, without indicating which were tuberculous and which were not tuberculous The very sick patients were quickly recognized by the rapid hemolysis All who were running any degree of increased temperature from tuberculous infection were separated from the others

This is very interesting, because the deproteinizing of the serum and antigen makes the separation possible, and because those showing the positive complement-fixation tests are the nontuberculous patients Those that show the most rapidly negative complement-fixation tests are the sickest from tuberculosis

Deproteinized guinea pig serum leaves the complement in protein-free solution This keeps longer and acts better

Antitoxic sera for therapeutic use are materially improved The removal of the protein takes away the element that produces anaphylactic shock, with the violent skin reactions that too frequently follow their use The protein-free antibody solution is not irritating or toxic Its physiologic action is quicker It is possible to precipitate out the antitoxic element by rendering the alkaline solution slightly acid The precipitate can then be dissolved in a minimum amount of very slightly alkaline physiologic salt solution

CONCLUSIONS

- 1 The deproteinizing of blood serum by electrolysis is practical and not difficult
- 2 It separates and removes the total protein from the toxic or antitoxic elements of the serum
- 3 The antitoxic elements act in their specific manner more precisely after the protein is removed

4 The antitoxic element with the protein removed keeps better and tolerates a higher temperature

5 The removal of the protein from the serum by electrolysis makes the complement fixation test more reliable

6 It makes the laboratory detection of tuberculosis possible from the blood serum

7 It removes the objectionable, and at times dangerous, element from antitoxic sera

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STUDIES ON LACTIC ACID IN THE BLOOD*

I THE EFFECT OF GLUCOSE AND INSULIN

By HARRY KOSTER M.D., F.A.C.S., M. GOLDZIEHER, M.D., W. S. COLLENS, M.D.,
AND I. E. GERBER, M.D. BROOKLYN, N. Y.

IN A RECENT paper we¹ demonstrated that in the postoperative state there is no evidence of the utilization of glucose, intravenously administered, using the respiratory quotient as an indicator. Since this glucose disappeared from the blood stream without any evidence of its oxidation, the question concerning its fate arose. Among other possibilities we considered its conversion into lactic acid. A review of the literature discloses the following facts:

Collazo and Lewicki² showed a rise in lactic acid after administration of glucose. Katayama and Kilian³ confirmed their results. The latter also obtained a rise following the administration of glucose and insulin. Briggs and his coworkers⁴ showed a rise in lactic acid with a drop in the blood sugar by the use of insulin alone. Collazo and Supniewski⁵ confirmed these last observations. Tolstoi, et al.⁶ obtained similar results inconstantly.

Contradictory results were obtained by other observers. Isaac and Adler⁷ found no change in lactic acid following insulin and glucose. Mendel, Engel and Goldscheider⁸ were unable to obtain a rise in lactic acid following glucose or insulin. Best and Ridout⁹ did not find a rise following insulin injections. C. F. Cori,¹⁰ Best and Scott¹¹ and Servantie,¹ working independently, arrived at the same conclusion. Otto Jervell¹² found no rise following carbohydrate feeding.

These conflicting results led us to make the following studies:

The effect of intravenously injected (a) glucose (b) insulin, and (c) glucose and insulin on the lactic acid level in the blood.

METHOD

These experiments were all performed on nondiabetic adult patients. The lactic acid determinations were done according to the method of Friedemann.

From the Research Laboratories of the Crown Heights Hospital, Brooklyn, N. Y.
Received for publication February 27, 1930.

Cotomo and Shaffer¹⁴ The blood was drawn without stasis, using sodium oxalate to prevent clotting. The proteins were precipitated according to the method of Fohn and Wu, and the filtrate was divided into two portions. On one portion quantitative blood-sugar determinations were made. The other portion was treated with 10 per cent CuSO_4 and 5 per cent Ca(OH)_2 to remove sugar and other interfering substances, such as creatinin and uric acid. This desugared filtrate was then used for the determination of lactic acid. Two determinations were made on each blood specimen and were found to check within the range of 5 per cent error for the method. Our results, in each case represent the average of two determinations.

The insulin and glucose were given intravenously. The amount of glucose used was 250 cc of a 20 per cent solution.

RESULTS

Group I Effect of glucose—In this series as shown in Table I, after the intravenous administration of glucose and the determinations of lactic acid made at hourly intervals for four hours, there was no rise found in the blood lactic acid level. In several instances there was a slight decrease.

TABLE I
THE EFFECT OF INTRAVENOUS GLUCOSE

	W W		B N		M G		S S		H H	
	SUGAR	LACTIC ACID	SUGAR	LACTIC ACID	SUGAR	LACTIC ACID	SUGAR	LACTIC ACID	SUGAR	LACTIC ACID
	MG	100 C C	MG	100 C C	MG	100 C C	MG	100 C C	MG	100 C C
Fasting	80	33.08	141	42.35	91	20.14	122	16.14*	93	23.29
Glucose	50 grams		50 grams		50 grams		50 grams		50 grams	
1 hour	129	17.44	156	22.05	100	17.10	148	21.59	136	19.80
2 hours	65	21.60	113	25.43	69	8.55	163	15.72	115	12.26
3 hours	77	22.95	60	19.74			141	15.22	79	16.65
4 hours	71	21.60	73	28.67	98	15.44	127	15.45	73	13.50

*Following the intravenous glucose this patient had a mild chill lasting about twenty minutes. This is the only patient in whom we obtained a rise in lactic acid immediately after glucose.

Group II Effect of insulin—With insulin alone, determinations made at half-hourly intervals for a period of two hours showed no significant changes in the blood lactic acid content, the variations being within normal limits.

TABLE II
THE EFFECT OF INTRAVENOUS INSULIN

	P P		M S		S F	
	SUGAR	LACTIC ACID	SUGAR	LACTIC ACID	SUGAR	LACTIC ACID
	MG	100 C C	MG	100 C C	MG	100 C C
Fasting	82	13.50	87	15.75	92	22.68
Insulin	12 units		12 units		15 units	
15 min	62	16.65				
30 min	38*	14.40	47*	16.87	31*	24.53
60 min	61	11.68	54	17.55	39	29.70
90 min	73	10.35	83	17.37	43	31.72
120 min	81	14.18	85	12.26	65	24.30

*Hypoglycemic reaction: Sweating weakness headache dizziness and restlessness. No convulsions or tremors.

Group III Effect of glucose and insulin—In this series, where insulin was given one half hour after intravenous glucose injection and determinations were made at hourly intervals for a period of three hours, there were also no significant changes in the blood lactic acid level

TABLE III
THE EFFECT OF INTRAVENOUS GLUCOSE AND INSULIN

	H B		N M	
	SUGAR	LACTIC ACID	SUGAR	LACTIC ACID
	MG	100 CC	MG	100 CC
Fasting	03	12.94	79	14.40
Glucose	40 grams		50 grams	
30 min	544	12.15	158	16.65
Insulin	12 units		15 units	
30 min	56	0.12	34	12.60
1 hour	65*	12.38	50*	15.75
2 hours	80	14.63	60	12.66
3 hours	82	11.86	75	15.98

Hypoglycemic reaction characterized by sweating weakness dizziness headache pallor and restless ss Patients complained of thirst and dryness of the throat. No convulsions or tremors

Group IV Effect of glucose and simultaneous respiratory quotient determinations—Following the administration of glucose and the determinations of lactic acid one hour later there was no rise in the lactic acid blood level. Concomitant respiratory quotient determinations showed the characteristic rise

TABLE IV
THE EFFECT OF INTRAVENOUS GLUCOSE WITH SIMULTANEOUS R/Q DETERMINATIONS

	FASTING			TIME AFTER GLUCOSE	AFTER GLUCOSE		
	SUGAR	LACTIC ACID	R/Q		SUGAR	LACTIC ACID	R/Q
	MG	100 CC			MG	100 CC	
P K	106	20.48	0.81	50 min	239	19.46	1.00
E M	92	18.70	0.68	60 min	216	22.21	0.80
D G	88	15.87	0.70	40 min	238	19.57	0.93
A S	93	29.25	0.74	45 min	184	27.80	0.86

CONCLUSIONS

There is no rise in the lactic acid level after the intravenous administration of glucose, insulin, or both. During the period of these observations glucose was oxidized as shown by the rise in the respiratory quotient. It would seem that lactic acid is not an intermediary stage in the metabolism of intravenously administered glucose.

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THE EFFECT OF ALLERGIC REACTIONS ON THE COURSE OF NONALLERGIC DISEASES*

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THE victim of bronchial asthma, hay fever, or urticaria, consulting a physician, presents an acute, sometimes almost tragic, situation which requires immediate relief. His only interest at the time is the one acute condition and the doctor's immediate aim is the relief of the active emergency. His next aim is the finding and removal of the exciting causes. Fortunately, since the development of the concept of protein sensitization as a cause for these allergic diseases, relief, entire or partial, is obtained in a large number of instances. As a consequence, the man who has developed a special interest and proficiency in allergy soon finds himself busy with this type of condition alone. The next logical development is the allergy specialist who treats only allergic diseases. There are vast numbers of asthma, hay fever, and urticaria victims, and the exclusive allergist accomplishes untold good in relieving these suffering hosts.

But we should bear in mind that allergy is but a branch of medicine and that it must not always be considered as a disease apart from other medical maladies.

Balyeat¹ has indicated that the sufferer from allergy is likely to be otherwise in exceptionally good health and has suggested that in some way the allergic state may offer a measure of protection against some of the other diseases. The element of heredity appears to be linked up in this situation. Nevertheless, while the proportions may be smaller, my experience has been that the allergic patient may be the victim of almost any of the other diseases which fall within the domain of internal medicine. I have not yet had an opportunity to make a statistical survey of my allergic series with this point in mind except with regard to blood pressure findings.

Hypotension is found in allergic patients often enough to have some diagnostic significance. Five per cent of my total series of office cases have hypotension. Twenty per cent of my allergic patients show low blood pres-

*Read before the Association for the Study of Allergy Portland Ore July 9 1929

sure readings. But a larger proportion of my allergic patients have normal blood pressures and some even suffer from hypertension.

While startling therapeutic results are often obtained in the allergic diseases by specific allergic treatment or avoidance alone, there is a definite proportion of patients who do not respond to these methods. The writer² and Peshkin³ have called especial attention to these groups and emphasized that other pathologic factors, nonallergic may influence the allergic reaction and its response to treatment. In connection with this I proposed a term, "allergic balance" or "allergic equilibrium" to designate the state of an allergic individual who although in contact with an allergen to which he is sensitive, remains symptom free. It has been shown that this allergic balance may be overthrown, with the precipitation of an allergic attack, either by the addition of more extensive allergic contacts with one or several allergens, or by the activity of some additional nonallergic factor.

The concept which I wish to bring out in this contribution differs distinctly from the foregoing in that the disease from which the patient is suffering is primarily a nonallergic organ or systemic disease. An allergic reaction which would otherwise be insignificant with this patient or might not even give rise to symptoms, will increase the disability from the primary nonallergic disease. The first case to be cited will be open to argument in that there may be a question as to which is the dominant disease manifestation, the allergic or the nonallergic, but the others appear to be clear cut examples of the effect of allergic reaction on the course of nonallergic disease.

CASE 1—Mrs W. P. aged twenty six years, complained of eczema of the hands. She was tested out with the various food and epidermal proteins and gave borderline reactions only to the proteins of sweet potato, squash, onion, cottonseed, wheat, and milk. After nearly three months of protein elimination reinforced by nonspecific methods, such as peptone injections, hypertonic saline intravenously and acinotherapy, there was no definite improvement, and a basal metabolism determination was made. This test was done not because the patient was presumably an allergic who had not responded to other methods but because she looked like a hypothyroid case. Cerebration was a little slow, the hair was rather coarse and the skin was definitely thick and pasty, giving a low grade myxedematous appearance to the face.

The metabolic rate was found to be minus nine around the lower limits of normal. The patient was placed on increasing doses of thyroid extract running up to four grains daily, and her metabolic rate increased on successive readings to minus seven, plus five and plus sixteen. Within ten days after the institution of thyroid therapy the eczema commenced to improve and in the third week it had cleared up entirely.

Through this time she had remained on her protein avoidances. After the hands had been free from eczema for over two weeks, she started using a prepared lard in cooking, one which contained cottonseed oil. The following day her eczema had returned. She then changed to pure hog lard and the hands promptly cleared up. She has had one recurrence since that time following the use of a different soap which may have been made from cottonseed oil.

Here is a case of hypothyroidism in which allergic eczema followed contact with specific allergenic proteins. Dermatitis occurs not infrequently in hypothyroidism and this raises the question whether the dermatitis is an integral part of the hypothyroidism or whether it may at times be due to a concomitant allergy. The man who is treating dermatitis in a myxedema case, with thyroid extract, must bear in mind that allergy may be a factor.

CASE 2—Miss P M History of scarlet fever in childhood No allergic history At the age of twenty three this patient's blood pressure was found to be elevated The course of her illness followed in general that of other similar cases For a few years she had a fluctuating hypertension By the age of thirty one it had become quite fixed around 170 and gradually increased to around 190 to 200 Occasionally it was higher There was only moderate arteriosclerosis but considerable cardiac hypertrophy By the time she was thirty four she suffered her first acute cardiac upset with circulatory collapse, extrasystoles, and pulsus alternans After a period of rest she was again back on her feet, but within thirteen months she began to have attacks of nocturnal dyspnea

These appeared to be primarily cardiac She would awaken suddenly with gasping and belching There was no premonitory cough, no typical asthmatic wheezing, and there was not the typical asthmatic history of relief with the raising of increased amounts of mucus The pulse was rapid but regular during the attacks They passed off fairly rapidly, after the belching of large quantities of gas

Although the case appeared to be a clear cut example of true cardiac asthma, sensitization tests were performed and the patient was found to be clearly sensitive to feathers She was also found sensitive to the proteins of egg, corn, mackerel, lobster, and mustard She substituted silk floss pillows for feather pillows and remained free from these attacks of apparently cardiac asthma for nearly four months

At the end of this time cardiac failure commenced The systolic blood pressure fell from its level around 200, down to 138 The gas attacks at night recurred and were accompanied by cardiac pain and palpitation They came with increasing frequency so that five months later she was practically confined to her bed with congestive heart failure and attacks of acute pulmonary edema She died in her thirty seventh year

The attacks of acute pulmonary edema were altogether typical They began with palpitation and shortness of breath, and sometimes precordial pain, which within a few minutes was followed by the raising of large amounts of frothy, bloody, serous fluid This sputum was not at all that of an asthmatic It is of especial interest that ephedrin always promptly relieved the attacks Ephedrin had not, so far as I know, previously been tried out in acute pulmonary edema, but I have since used it in a case in which allergy did not appear to play a part, without benefit

The generally accepted treatment of acute pulmonary edema due to cardiac failure consists of the injection of morphine and atropine Adrenalin has been recommended, but some authorities are very positive in the statement that adrenalin makes the condition worse

This patient obtained much better results from ephedrin than from morphine and atropine

These observations strongly suggested that an acute allergic reaction at times brought on attacks of acute pulmonary edema associated with cardiac failure One additional incident lends strong corroborative evidence This patient had observed that in one movie theatre in her home town, a rather small, poorly ventilated one, during the last few months before she became bedridden, she would within fifteen or twenty minutes develop acute shortness of breath, dyspnea, and palpitation, and would soon be coughing up bloody froth This was the only building in which the phenomenon occurred Indeed with this one exception the attacks occurred practically always at night after retiring I had the opportunity of observing her during one of these attacks which came on at the movie and which rapidly cleared up within half an hour after leaving the place and within fifteen minutes after the administration of fifty milligrams of ephedrine by mouth That the phenomenon was not associated with eye strain or some other possible factor is indicated by the fact that she could go into other cinemas without trouble She was not sensitive toorris root

CASE 3—B S, a girl seven years old, with moderately advanced pulmonary tuberculosis was under treatment at Blue Ridge Sanatorium, Charlottesville, Va She was sensitive to timothy, orchard grass, and ragweed She was admitted during the winter and manifested rapid improvement in her tuberculous infection Serial x rays showed corresponding evidence

of improvement With the onset of the pollen season her asthma reappeared, accompanied for the first time in several months by fever The next roentgenogram showed a definite reactivation, with increase in the distribution of the tuberculous process Following subsidence of her asthma the temperature again returned to normal

DISCUSSION

These 3 cases scarcely require prolonged discussion Their significance appears to be self evident Certainly in the second and third, acute allergic reactions exerted a detrimental effect on the course of the chronic, nonallergic diseases It seems reasonable to presume that other nonallergic diseases than those mentioned might also be so influenced

Many observers have suggested that allergy plays a part in migraine In 1927, I presented⁴ the evidence from my clinical experience which seemed to demonstrate conclusively the presence of an allergic factor in migraine There are some who maintain that biliary tract pathology or duodenitis or colonic disease or some obscure central nervous system defect, as the case may be, is the primary cause for migraine Certain it is that in migraine there is often close association with biliary tract disease and probably also with duodenitis Now it really makes no great difference which is the primary underlying pathology, allergy or the abdominal condition Apparently one may influence the other

In 1922, I described my first case of mucous colitis in which there appeared to be an allergic factor In 1927, Hollander⁵ described 5 cases and, in 1928, I added 6 more to my own series⁷ In this communication I brought forth evidence that allergy is at least one factor in the causation of mucous colitis I emphasized that certainly, at least in the well developed cases, it is not the only factor, since such conditions as constipation, infection, food outrage, needless surgery, irritating enemas and the like serve to exaggerate the local pathology

I have presented elsewhere what I consider excellent evidence that some cases of mucous colitis as well as migraine are primarily allergic But for the present discussion it makes little difference whether they are basically allergic diseases, or fundamentally nonallergic In either case allergy often colors the picture and not infrequently exaggerates the symptoms But in the case of chronic myocarditis and chronic pulmonary tuberculosis, the allergic reaction appears to be extrinsic to the basic pathology Sensitization to the protein of the tubercle bacillus need not enter into the present discussion

In conclusion, I would say that this paper is presented, first as a plea to the man who is limiting himself strictly to allergy calling his attention to the possibility of allergic factors in other maladies, and second, as a plea to the general physician and other specialists, reminding them that inasmuch as 10 per cent of all people appear to be allergic in one way or another allergy is a condition which sometimes must be brought into consideration in the treatment of apparently nonallergic diseases

Allergy is not strictly speaking a specialty Like tuberculosis, cardiology, and gastroenterology, it is but a subdivision of internal medicine, and for most useful accomplishment must not be too widely separated from the more general subject

Balyeat⁸ has placed before us the need for more thorough undergraduate instruction in clinical allergy in our medical schools. The cases here reported illustrate how an internist might serve his patients better by virtue of a more thorough understanding of allergy and its synergistic potentialities.

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THE RÔLE OF BACILLUS WELCHII IN PERNICIOUS ANEMIA*

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FOR many years the intestinal canal has been searched for the cause of pernicious or idiopathic anemia. Various organisms have been considered and rejected. Most important of these are the B coli and streptococcus groups and more recently anaerobes, particularly *Bacillus welchii*. A possible etiological relationship has been inferred from the greatly increased numbers of these organisms in the intestinal tract, and their appearance at higher levels in the upper small intestine, which is relatively free from bacteria in the healthy individual.

In the *Journal of Pathology and Bacteriology*, July, 1928, Davidson shows that very high counts of *B welchii* are obtained in this disease. He employs a fecal suspension of definite opacity in decimal dilutions ranging from 1 in 10 to 1 in 100 million, distributed in Tubes No 1 to No 9. These tubes are plated to a ferrous sulphate sodium sulphite glucose agar medium for the growth of anaerobic bacilli in the vegetative stage. Anaerobes reduce the sulphite to sulphide, producing black colonies. We have examined a large number of these colonies and confirm Davidson's finding, that a large proportion of black colonies (over 90 per cent) contain *B welchii*. He finds that Tube 3 (dil 1 in 100) represents the upper limit from which *B welchii* is isolable in healthy persons, while in 33 of 41 patients suffering from pernicious anemia, *B welchii* was isolated from Tube 9.

His technic has been closely followed for the sake of comparison in a survey of the feces of healthy Egyptians. Over one hundred prisoners in a local jail, in good health, on a homogeneous diet, largely vegetable, and having had no antecedent bowel complaint were the subject of examination. The *B welchii* content only was considered.

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We have found that 21 per cent of these show B welchii in very high dilutions, i.e., between Tubes 3 and 9, and most of them in and nearer 9 than 3. In other words they are in such quantity as to occupy a pathologic zone in terms of Davidson's standard.

A point of interest in this connection is that pernicious anemia is a very uncommon disease in Egypt compared with its incidence in England.

Recently one of us (W. L. F.) has investigated the stools of healthy Egyptians in relation to their content of B. tetani and found these exceedingly rich in their yield of this organism.

We would conclude that the stools of healthy Egyptians have an anaerobic standard different from that reported for others by Davidson.

STUDIES IN THE PHARMACOLOGY OF LOCAL ANESTHETICS*

III. COMPARISON OF GAMMA (2 METHYL PIPERIDINO) PROPYL BENZOATE HYDROCHLORIDE WITH COCAINE AND PROCAINE ON EXPERIMENTAL ANIMALS

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INTRODUCTION

PRELIMINARY pharmacologic data on certain of McElvain's¹ local anesthetics have been reported by Jones,² and Rose,^{3, 4, 6, 7} and published by McElvain and his associates. Further physiologic investigations show several of these compounds to be outstanding when toxicity, duration of anesthesia, lack of irritating properties, and potentiation by adrenalin are considered. They are soluble in water, their solutions stable, and they are readily sterilizable by heat. After it had been ascertained that they possessed the properties of a good local anesthetic,⁸ they were submitted to Dr. Wm. R. Meeker of Mobile, Alabama, for comparative evaluation. Dr. Meeker conducted the tests upon himself, and in a personal communication stated that gamma (2-methyl piperidino) propyl benzoate hydrochloride (hereafter designated as No. 33) was the best of those studied by him.

Further and more detailed study of this one compound was required. Some of the results of that work are presented here. To obtain an idea of the value of No. 33 in comparison with substances with which the clinicians have had wide experience, a number of pharmacologic tests have been made, using cocaine and procaine parallel with the new local anesthetic. The data included are results obtained from this work.

METHODS OF EVALUATION

Duration of anesthesia and toxicity are the chief factors influencing the value of any local anesthetic substance. Toxicity data involve only animal

experimentation whereas duration has been determined by the use of both animal and human subjects. To prevent confusion, only the work done on animals will be included in this article. Clinical data and the accumulated experiments on human subjects will appear later.

1 *Duration of Anesthesia*—The duration of anesthesia on mucous membranes was determined by the method described by Schmitz and Loevenhart.⁹ An aqueous solution of the drug to be tested was instilled into the pouched lower lid of a rabbit's eye and the cornea bathed for one minute. After the remaining solution was drained off, stimulation was given the cornea by application of a dull-pointed glass rod. No effort was made to distinguish between partial or deep anesthesia, duration being judged as the time intervening between the loss of the wink reflex and its return.

Intracutaneous infiltration duration was determined by the method described by Rose.¹⁰ Injections of given doses of solutions of equal percentage were made on the closely clipped backs of guinea pigs. The wheals thus formed were stimulated by means of an induced electric current. Any movement of the animal due to the stimulation was considered the end-point of duration.

2 *Toxicity*—Intraperitoneal toxicity values on rats were the result of injections made halfway between the sternum and the symphysis pubis in the midline. This test was done in an attempt to obtain some idea of the toxicity of these compounds when used in the urinary tract, since the vascularity of the peritoneum and its contents most nearly approaches that of the urethra and bladder. A direct determination by injection into the urinary tract of experimental animals was not practical because it could not be done without operative procedure. This would have involved the use of general anesthesia, a factor which influences the toxicity of local anesthetics greatly.

Intravenous toxicity experiments were done on rats and rabbits. For the rats, the intravenous injection method of Roth¹¹ was used. Injections were made at the rate of fifteen to eighteen seconds for every one-tenth of a cubic centimeter of solution,¹² in order that the speed of the injection might not be a factor in increasing the toxic effect of the local anesthetics. The same point is of extreme importance in every case of intravenous injection and was observed very carefully in the work with rabbits. The lateral marginal vein of the ear was the portal of entry in the case of this animal.

Subcutaneous injections for toxicity determinations on mice were done after the method of Schmitz and Loevenhart.¹³ The injections were made in the abdominal region as near the lower margin as possible and in volume doses never exceeding one-half of a cubic centimeter. The same general method was followed in the case of white rats.

DATA

Table I shows the duration of anesthesia upon the rabbit's cornea when the local anesthetic is in concentrations of 1 and 2 per cent in aqueous solutions, and the duration of anesthesia intracutaneously in the guinea pig, in doses of one-tenth of one cubic centimeter of a 1 per cent solution.

For the rabbit's cornea anesthesia values given here for cocaine correspond well (within experimental limits) to those already recorded. The literature gives for 1 per cent solutions eighteen minutes,¹⁸ eighteen and twenty minutes,¹⁶ and for 2 per cent solutions twenty seven minutes,¹⁶ twenty eight minutes,¹³ twenty nine minutes,³ 'thirty minutes'¹⁸ and thirty two minutes.¹⁹

TABLE I*
DURATION OF ANESTHESIA (EXPRESSED IN MINUTES)

ANESTHETIC	INTRACUTANEOUS INFILTRATION GUINEA PIGS	RABBIT'S CORNEA	
	1%	1%	2%
Cocaine	31	24	35
Procaine	24	no action	no action
No. 33	44	22	34

*The figures for this table are based on the results obtained from not less than 20 animals in each case. For cocaine 1 per cent solution on rabbit's cornea the number of animals used was 10. 2 per cent solution 24. Six of the latter group gave the duration value of sixty minutes reported in the second paper of this series. (J. LAB. & CLIN. MED. 15, 239, 1929.)

Cocaine anesthesia by subcutaneous injection on a dog's back is reported by Pittenger,¹ as being eleven times more effective than procaine. Meeker,¹⁹ using the same method and doses of 0.25 cc. of 1 per cent solutions, found the duration of cocaine anesthesia to be thirty minutes, and procaine, eighteen minutes. In the concentrations used, and when in contact with the rabbit's cornea for one minute procaine produces little or no anesthesia.

The data found in Table II are toxicity test results expressed in terms of the "median lethal dose." Considerable confusion seems to exist as to the meaning of "minimal lethal dose" so long employed as an expression of toxicity. It is considered by some authors as the amount of a drug just sufficient to kill an animal occasionally, that which kills 50 per cent or that which is just large enough to kill all the animals on a given dose.⁶ The term "median lethal dose," which means the dose that will kill 50 per cent of a large* group of animals, has been decided upon as giving the fairest measure of toxicity values. This term together with its abbreviation L. D. 50 (lethal dose, 50 per cent) was suggested by Trevan.⁶

TABLE II
TOXICITY DATA IN TERMS OF L. D. 50

METHOD OF INJECTION	ANIMAL	COCAINE	PROCAINE	NO. 33
1 Intravenous	Rats	17.5	53	20
2 Intravenous	Rabbits	17.0	57	28
†3 Subcutaneous	Mice	150.0	800	800
†4 Subcutaneous	Rats	250.0	2100	1300
5 Intraperitoneal	Rats	70.0	300	120

†The subcutaneous injections in the case of mice and rats were repeated with adrenalin in dilution of 1:2,000, added to the anesthetic solutions. The L. D. 50 for cocaine, procaine and No. 33 with adrenalin on mice was 150, 800 and 800 milligrams per kilogram respectively. On rats the results were (in the same order) 110, 2800 and 1500 milligrams per kilogram.

The values given above for cocaine and procaine agree within the limits of experimental error, with those reported in the literature to date. Cocaine

toxicity per kilogram of body weight by intravenous injection on white rats is reported as 125 mg^{21 22} and 175 mg³, and on rabbits, 75 mg²³ injected fairly rapidly

Toxicities for cocaine by subcutaneous injections on white mice are reported as 150,^{13 24} 200-350²³ and 250 mg per kilogram³, and on rats, 200 300²³ and 250 mg per kilogram¹³

For procaine, the intravenous injection results recorded on rats are 50,³ and 45 to 55 mg per kilogram,^{21 22 23} and on rabbits, 40 to 70²³ and 55 to 65 mg per kilogram¹³

Toxicities, by subcutaneous injection of procaine on mice, are stated by other workers to be 900^{24 13} and 1,000 mg per kilogram,³ and on rats, 1600 to 2000²³ and 1600 mg per kilogram¹³

THERAPEUTIC INDICES

Therapeutic indices were calculated according to the method and formula of Schmitz and Loevenhart,⁹ as follows The numerical duration value of a given local anesthetic multiplied by the numerical toxicity value of the same substance is divided by a number derived in the same way from the standard Cocaine taken as the standard has the value of unity The other substances which are to be compared may be referred to in terms of cocaine

Letting "R" represent the anesthetic which is to be compared with the standard "S," the formula reads as follows

$$\frac{\text{R duration of anesthesia by R toxicity}}{\text{S duration of anesthesia by S toxicity}} = \text{Therapeutic Index}$$

All data in Table III were derived from Tables I and II

TABLE III
THERAPEUTIC INDICES

ANESTHETIC	METHOD	PER CENT SOLUTION	NUMBER'S CORRESPOND TO TESTS IN TABLE II					
			1	2	3	4	5	AVERAGE
Cocaine	Rabbit's cornea	1	1 00	1 00	1 00	1 00	1 00	1 00
No 33	Rabbit's cornea	1	1 05	1 51	4 90	4 77	1 57	2 76
Cocaine	Rabbit's cornea	2	1 00	1 00	1 00	1 00	1 00	1 00
No 33	Rabbit's cornea	2	0 75	1 60	5 18	5 05	1 66	2 85
Cocaine	Guinea pig	1	1 00	1 00	1 00	1 00	1 00	1 00
No 33	Guinea pig	1	1 62	2 34	7 56	7 38	2 44	4 27
Procaine	Guinea pig	1	2 52	2 60	4 13	6 51	3 22	3 80

SUMMARY

By Table I it is shown that No 33 is equally as active as cocaine in 1 and 2 per cent solutions on the rabbit cornea, and twice as active as procaine and one and one-half times as active as cocaine by the guinea pig infiltration method

Table II shows that No 33 is less toxic than cocaine in every case using five different methods of administration It is more toxic than procaine by all methods Subcutaneously on rats it shows a wide margin of safety

The therapeutic indices derived from the data in Tables I and II and shown in Table III give the advantage to No 33 over cocaine in 1 and 2 per

cent solutions on the rabbit's cornea in nine cases out of ten considered. By the guinea pig method, No 33 has the advantage over procaine in two of five cases. It is to be noted however that the average of the five shows a result in favor of No 33. The numerical values of No 33 in this group of indices are higher than those of cocaine in every instance.

CONCLUSIONS

1 No 33, gamma (2 methyl piperidino) propyl benzoate hydrochloride, has been compared pharmacologically with cocaine and procaine.

2 No 33 is much less toxic than cocaine when given by subcutaneous injection.

3 While being more toxic than procaine, No 33 still has a great margin of safety when administered subcutaneously.

4 No 33 is active by topical application to mucous membranes as well as by infiltration.

5 No 33 compares favorably with the recognized local anesthetics, cocaine and procaine.

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LABORATORY METHODS

THE TECHNIC OF DETERMINATION OF THE RELATIVE MASS, THE INDIVIDUAL CELL VOLUME, AND THE VOLUME INDEX OF THE ERYTHROCYTES OF MAN*

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THE determination of the size of the erythrocyte is of increasing importance in clinical medicine. Many examples of the value of such data might be given. To mention only one, in pernicious anemia, an accurate determination of the size of the red cells is necessary for diagnosis and the one most valuable indicator of the course of the disease. The widespread interest in the subject of red cell size is best shown by the increasing amount of research concerning it.

One measure of the size of the erythrocyte is its relative diameter. The pioneer work of Price-Jones¹ on the red cell diameter in health and disease has stimulated much research from this angle. The measurement of diameter only does not take into account the thickness of the cell, and relatively few cells are measured in practical clinical work. It seems far preferable to determine, as a measure of size, the total cell volume since this includes all dimensions.

The determination of volume involves first of all an accurate estimation of the relative mass of corpuscles in a given volume of blood, or of the volumetric relationship between corpuscles and plasma. The relative mass is the number of c.c. of corpuscles per 100 c.c. of blood when the corpuscles without alteration in volume are packed closely so that no plasma remains in the interstices of the cells.

The red corpuscles are composed of a semisolid hemoglobin-containing material which at the surface is dense and membrane-like. They are elastic and flexible, changing form and volume easily under different conditions. Variations in the electrolyte content of the surrounding medium quickly cause changes in the cell volume. Proper packing to eliminate fluid between the cells requires sufficient time and power of centrifugation.

The estimation of the average individual cell volume necessitates also an accurate count of the red cells of the blood for which the relative cell mass is being determined. The volume index is only a comparison of the individual cell volume of the unknown blood with the known normal individual cell volume. The two procedures involved are the estimation of the relative mass per 100 c.c. or the volume percentage of red cells and the estimation of the number of red cells per cm.

The relative cell mass is determined by the rapid centrifugation of unaltered blood or by centrifugation for a longer period of time of blood to which an anticoagulant has been added. The true volume is obtained only when the individual cell volume is unaltered and packing is complete. Numerous methods

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have been employed in determining the cell volume but few meet these requirements

I have summarized in Table I all the more important investigations on the determination of the red cell volume of man. Many other studies have been made but are not sufficiently complete to justify inclusion. The volume percentage of cells is much the same everywhere when determined by an accurate method. The number of red cells vary quite widely so the average individual cell volume shows quite marked variations. The summary in Table I shows the figures for men for different workers when an isotonic coagulant is used or when the necessary corrections are made for shrinkage due to use of a hypertonic anticoagulant.

Van Allen¹³ in describing a new hematocrit tube reviewed the important factors in the accurate determination of the red corpuscle volume. He found 13 per cent sodium oxalate isotonic with the blood of the rabbit but reported no studies with human blood. He used a capillary hematocrit in a centrifuge of 9 cm radius and centrifuged for fifteen minutes at 2700 rpm.

The different factors to be considered in the determination are (1) type of hematocrit, (2) the anticoagulant (3) the time and power of centrifugation.

TYPE OF HEMATOCRIT

The earlier investigators employed the hematocrit invented by Blir and first described by Hedin¹⁴. This is a straight capillary tube about 10 cm in length which is filled with unaltered blood and centrifuged at high speed for a few minutes. Only a small amount of blood is required and no anticoagulant is necessary. This is neither a satisfactory nor an accurate method and is now seldom used. The only extensive research work done with this instrument was that of Capps in 1905.

The Bonninger tube is a modification of the hematocrit of Hedin. It is a graduated capillary U tube which is filled with blood to which an anticoagulant has been added. This was first used by Bonninger,⁴ and later by Csaki,⁵ and by Froehlich.⁷ I have had no experience with this tube but see little to recommend it since a larger tube is far preferable.

Several other sizes of straight capillary tubes have been employed. These have usually been made and graduated in the laboratory in which they have been used. In addition to not being standard instruments these have all the disadvantages of the Hedin tube.

The best of the capillary tubes is that devised by Van Allen¹³. This enables the use of a standard centrifuge and fittings with an isotonic anticoagulant solution. It has the disadvantages of other micro methods. Results with this tube are not included in Table I since no data have been reported for human blood.

In my original work on volume index¹⁰ I employed a graduated 15 cc centrifuge tube using 10 cc of blood and 2 cc of the anticoagulant solution. This is a standard tube, can be easily accurately calibrated and used with the usual centrifuge fittings. It has the disadvantage of requiring 10 cc of blood and the results cannot be read quite so sharply as with a tube of smaller caliber. Kuhnel,¹¹ Osgood,¹² and Wintrobe and Miller¹⁴ used graduated tubes of 4 or 5

TABLE I
SUMMARY OF RESULTS OF DIFFERENT WORKERS ON DETERMINATION OF VOLUME OF THE RED BLOOD CELL

NAME OF OBSERVER	DATE	COUNTRY	TYPE OF HEMATOCRIT	TIME OF CENTRIFUGATION	ANTICOAGULANT	AVERAGE RED BLOOD CELL VOLUME PER 100 c c (= VOL UME PERCENTAGE OF CELLS)		AVERAGE RED BLOOD CELL COUNT	AVERAGE RED BLOOD CELL VOLUME FOR 5 MILLION CELLS	AVERAGE VOLUME OF INDIVIDUAL RED BLOOD CELL
						c c	men (+) and women (6)	millions	c c	cubic microns
Capps ²	1903	United States (Boston)	capillary tube (Hedin)	3 minutes at 10,000 r p m	none		men (+) and women (6) 48.0	4.83	50.00	100.00
Larrabee ³	1911	United States (Boston)	capillary tube (2 mm x 10 cm)	gravity sedimentation for 13 days	100 mg sodium oxalate to 10 c c blood		men (12) 53.0 women (9) 49.7	5.27 4.97	50.00	100.00
Bonninger ⁴	1919	Germany	capillary tube (Bonninger)	to constant volume (40 minutes) at 4000 r p m	hirudin		men (10) 46.4 women (10) 39.4	4.87 4.30	46.8	93.6
Csakis	1922	Hungary	capillary tube (Hedin)	7 minutes	none		men (11) 45.2 women (12) 44.0	4.94 4.81	45.8	91.6
Campbell ⁶	1922	England	capillary tube (size ?)	15 minutes	50 mg potassium oxalate to 10 c c blood		men and women (16) 40.00	5.15	38.8	77.6
Froehlich ⁷	1922	Germany	capillary tube (Bonninger)	30 minutes at 4000 r p m	hirudin		men (7) 45.0 women (13) 41.4	4.96 4.48	45.8	91.6
Bae and Mollers	1922	Norway	capillary tube (1 mm x 5 cm)	1 hour	defibrinated		men (10) 46.4 women (10) 38.7	5.53 4.74	41.2	82.6
Gram and Norgaards	1923	Norway	capillary tube (10 cm)	until transparent (1 1/2 hours) at not less than 3000 r p m	hirudin		men (10) 46.3 women (10) 40.5	5.45 4.65	43.4	86.8

*When corrected for shrinkage due to oxalate (0.7%) this figure becomes 40.5 c c

†When corrected for shrinkage due to oxalate (3.5%) this figure becomes 40.6 c c

TABLE I—CONT'D

NAME OF OBSERVER	DATE	COUNTRY	TYPE OF HEMATOCENT	TYPE OF CENTRIFUGATION	ANTICOAGULANT	AVERAGE RED BLOOD CELL VOLUME PER 100 CC. (= VOLUME PERCENTAGE OF CELLS)	AVERAGE RED BLOOD CELL COUNT	AVERAGE RED BLOOD CELL VOLUME FOR 5 MILLION CELLS	AVERAGE INDIVIDUAL RED BLOOD CELL
						cc	millions	cc	cubic microns
Haden ¹⁰	1923	United States (Kansas City)	15 cc graduated centrifuge tube	30 minutes at 2500 rpm	2 cc 1.6% sodium oxalate to 10 cc blood	men (40) 47.7 women (12) 41.0	4.98 4.26	48.0	96.0
Kuhnelt ¹¹	1925	Denmark	5 cc graduated tube	to constant volume (30 minutes)	1 cc 3% sodium citrate to 9 cc blood	women (10) only 41.5	4.74	43.7	87.4
Osgood ¹²	1926	United States (Portland Oregon)	4 cc graduated tube	to constant volume (4.1 hour)	20 mg potassium oxalate to 10 cc blood	men (94) 43.04 women (100) 41.0	4.40 4.80	43.0	86.0
Jorgensen and Warburg ¹³	1927	Denmark	thin tubes (size 1)	until transparent at 4000 rpm	defibrinated	men (3) and women (4) 45.5	4.30	43.0	86.0
Wintrobe and Miller ¹⁴	1929	United States (New Orleans)	4 cc graduated tube	to constant volume (4.1 hour) at 3500 rpm	40 mg potassium oxalate to 10 cc blood	men (100) only 43.4	5.85	37.1	74.2
Haden	1929	United States (Kansas City)	12 cc graduated centrifuge tube	1 hour at 2500 rpm	2 cc 1.4% sodium oxalate to 10 cc blood	men (7) and women (3) 44.8	4.86	46.1	92.2

TABLE II

	NO CASES	LOCATION	RED BLOOD CELLS PER 100 C.C. (= VOLUME PERCENTAGE OF CELLS)
Bonninger	10	Germany	46.4 c c
Csaki	11	Hungary	45.2
Froelich	7	Germany	45.0
Bie and Moller	10	Norway	46.4
Gram and Norgaard	10	Norway	46.3
Haden	20	United States	46.5
Osgood	94	United States	46.6
Jorgensen and Warburg	3	Denmark	45.5
Wintrobe and Miller	100	United States	46.5

c c capacity made from Mohr pipettes. These require smaller amounts of blood, but are not standard and require special calibration.

There is never any objection, even in the most anemic patient, to taking sufficient blood for a careful study. A macro method is necessary for accuracy. Specially made tubes of 4 or 5 c c capacity when accurately calibrated are perfectly satisfactory. I still prefer to use a standard 15 c c centrifuge tube with 10 c c of blood and 2 c c of isotonic anticoagulant. Here one is using sufficient blood for accurate reading in a standard piece of apparatus. 12 c c centrifuge tubes of pyrex glass are now available also and are somewhat more satisfactory than those of 15 c c capacity. I have used these 12 c c tubes exclusively in the experiments reported herewith. Each tube was calibrated by weighing with distilled water.

THE ANTICOAGULANT

The original Blx hematocrit was designed for use without an anticoagulant. No anticoagulant was used by Capps. There are only two anticoagulants which do not change the volume of the red cell and yet add no volume to the blood. These are heparin and hirudin. Hirudin was used by Bonninger, by Froelich, and by Gram and Norgaard.⁹ The other anticoagulants used are citrate and oxalate. Three per cent sodium citrate was used by Kuhnelt and by de Jong.¹⁷ The oxalates have been used in a number of ways. A method in common use is the addition of one drop of a saturated solution of potassium oxalate to each 5 c c of blood. Twenty mg of solid potassium oxalate per 10 c c of blood is often recommended. Campbell⁶ used 50 mg of solid potassium oxalate to 10 c c and Wintrobe and Miller 40 mg to 10 c c. Lairabee³ used 100 mg of solid sodium oxalate to 10 c c of blood.

Hooper, Smith, Belt, and Whipple¹⁸ pointed out that solid oxalate causes a marked shrinking of red cells. They suggested the use of 2 c c of a 1.6 per cent solution of sodium oxalate to 10 c c of blood for determining the volume percentage of red cells in the dog. This method was used in my original work in determining the volume index of the red cells of man. Later observers who have used solid potassium oxalate have recognized the fact that the cell volume is decreased with this procedure. Osgood states that the use of 20 mg to 10 c c causes a 3.5 per cent shrinkage. Wintrobe and Miller allow 6.7 per cent for shrinking on adding 40 mg of oxalate to 10 c c.

It is apparent that it is most unsatisfactory to attempt accurate results unless the cell volume is unchanged. I have made a series of experiments with human blood to determine the variation in volume with different anticoagulants. There were 100 cc of blood withdrawn with a large syringe from each individual and just enough hirudin or heparin added to prevent coagulation. With a calibrated pipette, 10 cc of blood was run into each of a series of 12 cc centrifuge tubes. The pipette and tubes were accurately calibrated by weighing with water on a chemical balance. One tube was taken as a control. To the other tubes the different anticoagulants were added as indicated. All tubes from one individual were centrifuged simultaneously as indicated in the tables.

In the initial experiment (Table III) different dilutions of sodium oxalate and one drop of a saturated solution of potassium oxalate were used. These showed the marked shrinkage due to use of the saturated solution of potassium oxalate. Two specimens of defibrinated blood gave results slightly higher than with hirudin alone. It is apparent from this preliminary experiment that the correct amount of sodium oxalate to prevent coagulation without changing the cell volume lies between 1.4 per cent and 1.6 per cent.

TABLE III

VOLUME PERCENTAGE OF RED BLOOD CELLS WITH DIFFERENT ANTICOAGULANTS
(CENTRIFUGATION FOR ONE HOUR AT 2500 R.P.M.)

NO	VOLUME PERCENTAGE OF RED BLOOD CELLS WITH							DEFIBRINATED ONLY
	HIRUDIN ONLY	10 CC HIRUDINIZED BLOOD WITH 2 CC SODIUM OXALATE					10 CC WITH 1 DROP SATURATED SOLUTION POTASSIUM OXALATE	
		12%	14%	16%	18%	20%		
1	44.5	46.0	45.0	44.0	43.0	43.0	41.0	47.0
2	44.0	45.0	44.0	43.0	43.0	42.5	38.0	45.0
3	49.0	50.0	50.0	48.5	47.5	46.5	44.0	—
Average	45.8	47.0	46.3	45.2	44.5	44.0	41.0	—

In the second set of experiments (Table IV) only three dilutions of sodium oxalate were used, namely 1.4, 1.5, and 1.6 per cent. The effect of adding solid sodium oxalate equivalent to that as contained in 2 cc of a 1.4 per cent solution was determined by adding 28 mg to 10 cc of blood. Other tubes contained 1 cc of a 3 per cent solution of sodium citrate, 20 mg of solid potassium oxalate, or 1 drop of a saturated solution of potassium oxalate. The cell volume with the solid sodium oxalate and potassium oxalate and the saturated solution of potassium oxalate are much the same and are all much lower than with the hirudinized blood. The readings with the 1.4 per cent sodium oxalate solution (2 cc to 10 cc of blood) are almost exactly the same as with hirudinized blood.

These observations together with succeeding ones have convinced me that the 1.4 per cent sodium oxalate is isotonic with human blood since it gives the same results as heparin or hirudin. On the other hand the addition of 1 drop of a saturated solution of potassium oxalate (equivalent to 25 mg of oxalate) to 10 cc of blood causes the volume of cells to shrink 8 per cent.

TABLE IV
VOLUME PERCENTAGE OF RED BLOOD CELLS WITH DIFFERENT ANTICOAGULANTS (2500 REVOLUTIONS PER MINUTE)

NO	TIME OF CENTRIFUGA TION HOURS	HIRUDIN ONLY	10 CC HIRUDINIZED BLOOD WITH 2 CC SODIUM OXALATE			10 CC HIRU DINIZED BLOOD WITH 28 MG SOLID SODIUM OXALATE	10 CC HIRU DINIZED BLOOD WITH 1 CC 3 PER CENT SODIUM CITRATE	10 CC HIRU DINIZED BLOOD WITH 20 MG SOLID POTASSIUM OXALATE	10 CC HIRU DINIZED BLOOD WITH 1 DROP SATURATED SOLU TION POTASSIUM OXALATE
			1.4%	1.5%	1.6%				
1	1	48.5	48.7	48.0	48.0	44.4	48.0	44.7	45.3
	2	47.5	48.2	47.0	47.0	44.4	47.0	43.7	44.3
2	1	47.5	47.2	46.0	46.0	43.9	47.0	43.7	43.0
	2	42.5	42.0	41.3	41.1	38.5	41.7	39.0	39.9
Average all readings	1	42.0	41.0	40.3	40.7	37.0	40.7	38.0	38.4
	2	41.5	41.5	40.3	40.7	37.0	40.7	38.0	38.4
		44.9	44.8	44.3	44.0	40.9	44.2	41.2	41.5

THE TIME AND FORCE OF CENTRIFUGATION

The time of centrifugation has varied widely with different workers. Unless an anticoagulant is employed, only a few minutes' packing is possible before coagulation takes place. It is necessary to pack the cells to constant volume. This usually requires about one hour. The centrifugal force of a centrifuge varies with the diameter and the speed. To compare different methods of centrifugation one must know both the speed and the length of the arm of the centrifuge. The centrifugal force of a centrifuge varies directly as the first power of the radius and as the second power of the speed. Thus the centrifugal force with the same r p m with a radius of 20 cm is twice that with a radius of 10 cm. If the radius is the same however, the centrifugal force is four times as great with a speed of 4000 r p m as with a speed of 2000 r p m.

The striking thing shown in Table I however is the relative constancy of the cell volume provided the proper anticoagulant is used even with varying methods of centrifugation. This suggests that complete packing does not necessitate great centrifugal force.

In determining the relative mass for calculating the volume index, it is only necessary to use the same procedure in each case. However, in calculating absolute numbers such as the individual cell volume it is necessary that no plasma remain in the interstices between the cells. This involves complete packing of the cells. This end point is difficult to determine. The usual method has been to observe the cell volume of different intervals and continue the centrifugation until the volume is constant. Koeppe¹⁹ thinks complete packing is best shown by translucency of the cell mass to transmitted light. This criterion has been utilized by some workers.¹³

I have made some experiments to determine the relation of the volume to the time and speed of centrifugation. The results are shown in Table V. All specimens were centrifuged and read at intervals of one half, one and two hours. The average of 12 determinations show the volume practically constant after an hour's centrifuging, so I have taken this time as the standard for comparison. The volume is only 2 per cent less with two hours' centrifugation than it is with centrifugation for one half hour. The centrifuge used was an International No 2 with a radius of 15 cm.

CALCULATION OF THE AVERAGE INDIVIDUAL CELL VOLUME

As early as 1867 Welch⁹ reported results on the size of the erythrocyte in terms of cubic microns. Numerous other workers have employed this most desirable unit of measurement. The only data needed for calculation are the relative cell mass and the red cell count. For instance from the data given in Table VI the average cell volume is calculated as follows. The average red blood cell count is 4 856 millions per c mm. The volume percentage of cells with 1.4 per cent sodium oxalate solution is 44.82 c c equivalent to 44.82×10^1 cubic microns. One hundred c c of blood with a red cell count of 4 856 million cells per c mm contains $4 856 \times 10^{11}$ cells.

The average individual cell volume is

$$\frac{44.82 \times 10^{12}}{4 856 \times 10^{11}} \text{cubic microns or } 92.1 \text{ cubic microns}$$

The individual cell volume in cubic microns is double the total cell volume per 100 cc of blood for 5 million cells per cmm. For instance if the count is 40 million per cmm and 100 cc of blood contain 36 cc of cells the total cell volume for 50 million cells is 45, and the individual cell volume is 900 cubic microns.

TABLE V

RELATION OF TIME OF CENTRIFUGATION TO VOLUME PERCENTAGE OF RED BLOOD CELLS WITH DIFFERENT ANTICOAGULANTS

NO	TIME OF CENTRIFUGATION AT 2500 R P M (HOURS)	VOLUME PERCENTAGE OF CELLS WITH			AVERAGE
		HEPARIN ONLY	2 CC 14% SODIUM OXALATE TO 10 CC. HEPARINIZED BLOOD	1 DROP SATURATED SOLUTION POTASSIUM OXALATE TO 10 CC HEPARINIZED BLOOD	
1	$\frac{1}{2}$	50.5	50.0	46.5	49.0
	1	50.5	50.0	48.5	48.7
	2	49.5	49.0	45.5	48.0
2	$\frac{1}{2}$	48.5	48.7	45.3	47.5
	1	47.5	48.2	44.3	46.7
	2	47.5	47.2	43.0	45.9
3	$\frac{1}{2}$	42.5	42.0	39.9	41.5
	1	42.0	41.0	38.4	40.5
	2	41.5	41.5	38.4	40.5
4	$\frac{1}{2}$	42.0	42.0	38.0	40.7
	1	42.0	41.0	38.0	40.3
	2	42.0	41.5	38.0	40.5
	$\frac{1}{2}$	Average of twelve determinations			44.7
	1	" "	" "	" "	44.1
	2	" "	" "	" "	43.7

TABLE VI

RELATION OF VOLUME PERCENTAGE OF RED BLOOD CELLS TO NUMBER OF CELLS (CENTRIFUGATION FOR ONE HOUR AT 2500 R P M)

NO	SEX	RED BLOOD CELL COUNT IN MILLIONS	VOLUME PERCENTAGE OF RED BLOOD CELLS WITH		
			HEPARIN ONLY	2 CC 14% SODIUM OXALATE TO 10 CC BLOOD	1 DROP SATURATED SOLUTION POTASSIUM OXALATE TO 10 CC BLOOD
1	M	4.80	44.0	44.0	40.5
2	M	4.83	44.5	45.5	41.5
3	M	4.93	47.0	47.5	42.0
4	F	4.30	40.5	40.0	38.5
5	F	4.38	42.0	41.0	38.0
6	M	5.45	50.5	50.0	45.5
7	M	5.60	47.5	48.2	44.3
8	F	4.64	42.0	41.0	38.4
9	M	4.85	47.5	47.0	42.5
10	M	4.78	44.0	44.0	42.0
Average		4.856	44.95	44.82	41.32

CALCULATION OF THE VOLUME INDEX

The volume index of Capps expresses the relationship between the number and size of the cells. It is calculated by dividing the volume percentage of cells in percentage of normal by the number of cells in per cent of normal. Example: The red cell count is 3,500,000 per cmm and 100 cc of blood contain 23.0

cc of packed cells Normal blood with a count of 5 million contains 46.0 cc of cells per 100 cc The volume percentage of cells in the unknown blood is $\frac{23.0}{46.0} = 50$ (volume percentage of normal) The red cell count is 70 per cent of normal The volume index is $\frac{50}{70} = 0.74$

THE TECHNIC OF DETERMINATION OF THE RELATIVE CELL MASS

Run 2 cc of 1.4 per cent solution of sodium oxalate into a graduated 12 cc or 15 cc centrifuge tube with a syringe Withdraw 20 cc of blood from an arm vein with a syringe Transfer exactly 10 cc to the centrifuge tube containing the sodium oxalate solution and mix by inverting Centrifuge for one hour at 2500 r p m in a large centrifuge Read the volume of cells The blood of normal young men yields about 46 cc of cells and of young women about 41 cc To the other 10 cc of blood add one drop of a saturated solution of potassium oxalate and mix This specimen is used for a red cell count and for hemoglobin determination

ILLUSTRATIVE CALCULATIONS

On centrifuging 10 cc of blood mixed with 2 cc of 1.4 per cent sodium oxalate at 2500 r p m for one hour 4.5 cc of packed corpuscles are obtained A normal blood with a count of 5 million yields 46 cc when similarly centrifuged The volume percentage of normal of the packed cells of the unknown blood is 98

The cell count of the unknown blood is 4,900,000

The average individual cell volume is

$$\frac{4.50 \times 10^3}{4.90 \times 10^{11}} = 920 \text{ cubic microns}$$

The volume index is

$$\frac{4.5}{4.6} = 1.00$$

SUMMARY

Experiments are reported to determine the best method of estimating the volume relationship between the red corpuscles and plasma of men

An accurate determination of this relationship necessitates the employment of an isotonic anticoagulant and sufficient power and time of centrifugation to completely separate the cells from the plasma

A 1.4 per cent solution of sodium oxalate is isotonic with human blood

Centrifugation for one hour at 2500 r p m with radius of 10 cm. will completely separate erythrocytes from plasma

The relative mass of the erythrocytes is best determined by centrifuging in such a way, 10 cc of blood to which 2 cc of 1.4 per cent sodium oxalate has been added The average red cell content of the blood of young men is 46 cc per 100 cc of blood and of young women 41 cc

Illustrative examples are given for the calculation of the volume index and the average individual cell volume

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I ON HEMOGLOBINOMETRY*

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OF THE routine tests used in diagnosis, the estimation of hemoglobin, necessary in the diagnosis of some of the commonest diseases, is one of the most indefinite. The methods used have wide individual errors beside those inherent in technic, there are unnecessary errors in manufacture of the instruments, and there is a justifiable uncertainty as to the normal standard. The direct methods of hemoglobin determination are not suited to routine use. Of the two systems against which the clinical instruments should be standardized, the determination by the oxygen capacity method of van Slyke has many pitfalls, although it is shorter than the exact ferrometric methods.

The hemoglobinometers in general use in this country have been the Tallqvist, Sahli, and the Dare, and recently the Newcomer. The color scale of the Tallqvist on comparison with the Newcomer is more reliable than generally stated, provided the book is kept closed except at the moments of use. A hemoglobinometer resembling the Tallqvist sent out broadcast to physicians by a commercial house, advertising a remedy doubtless valuable in anemia is marked 10 per cent higher for each color than the Tallqvist. The Sahli is also a simple method and its readings appear somewhat more exact than the Tallqvist, its color solution also keeps well when kept in the dark, but there are wide errors in technic and in manufacture (irregular diameter of tubes).

There are several objections to the Dare hemoglobinometer. Newcomer¹

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pointed out one of the most important—that there is no red glass whose light transmission curve at all corresponds to that of oxyhemoglobin that to obtain an exact match a red light would have to be used, and that the sensitivity of the eye for red makes it impossible to distinguish any but gross differences in color. Any one familiar with the Dare has noted the differences in reading between different observers due to their differences in color sensitivity, besides the difficulty of matching certain bloods and the effects of fatigue for red. Another source of error with the Dare not previously recorded is the darkening of the blood by CO. While venous blood is rarely used with the Dare capillary blood will invariably give higher readings than arterial, and especially is it higher in patients with poor aeration from any cause. After saturating blood with CO we obtained readings with the Dare of 35 gm Hb instead of 27 gm from the same specimen when oxygenated with similar variations up to 15.15 gm with CO against 13.2 gm oxygenated 30 per cent to 15 per cent higher than arterial blood. In the Fallqvist the blood is immediately oxygenated and this source of error does not exist. In addition with the Dare there is the objectionable semisecret feature of the depth of the pipette and the 15 per cent high reading for bloods over 65 per cent noted by Sanford not present in all instruments but seemingly common and due to insufficient grinding of the thicker half of the red glass prism. With these many sources of error it seems advisable to abandon the Dare hemoglobinometer.

The Newcomer glass disk has seemed the best hemoglobinometer for general adoption being accurate rapid and not expensive the B and L Newcomer costing little more than a Dare. By the purchase of a Newcomer disk alone at nominal cost the Duboseq or other colorimeter in use in every laboratory can be used as a hemoglobinometer. Because of the close correspondence of its light transmission curve with that of acid hematin the Newcomer disk is not only the best of clinical methods giving the same values for any eye but the only one giving readings approaching accuracy. It is not, however, an ideal method and has been modified several times. The original dilution of the blood was 1:250, the glass is now made to match against a 1:500 dilution (except the Klett instrument 1:400). The original advice to take the average of ten readings because of the 5 to 10 per cent variation has unfortunately not recently been insisted upon. The difficulty of matching certain bloods because of color (icterus) or turbidity (fat leucocytes) has been partly overcome by the addition by Bausch and Lomb of a dark blue glass filter, placed either in the lens system of the B and L hemoglobinometer or laid on top of the eyepiece when using a general laboratory colorimeter.

We had not questioned the general accuracy of the Newcomer disk until recently when we had occasion to examine a Klett colorimeter with a dark glass disk requiring according to direction a 1:400 blood dilution with which, however we got consistently higher readings. It is true, Klett says in the directions 'it is probably well to recalibrate the glass plates' but we felt that this was a matter which the clinical laboratory had a right to leave to the manufacturer, and it is certainly not practical for the average physician who desires careful hemoglobin readings on his patients. We therefore made

readings of bloods, the hemoglobin of which we measured by the van Slyke oxygen capacity method, comparing the readings of the B and L Newcomer (two instruments), dilution 1 500, and Klett, dilution 1-400

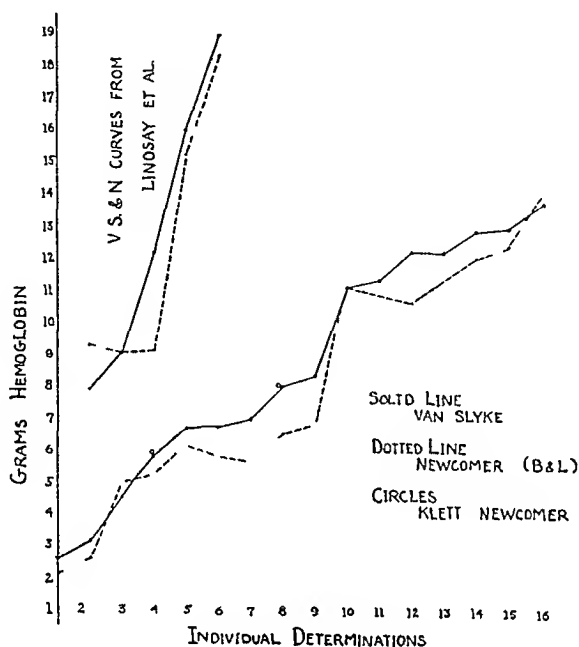


Chart 1

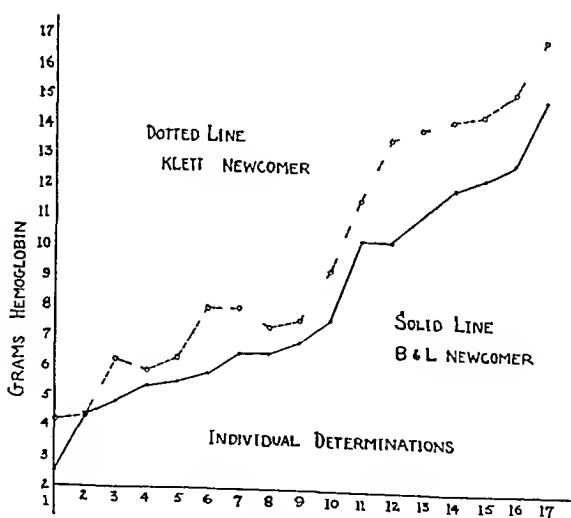


Chart 2

Chart 1 shows our results. Chart 2 is a further comparison of the B and L Newcomer and the Klett. It is recommended by van Slyke² that his oxygen capacity method be repeated on a specimen until results are constant. This we did with only a few of the determinations reported in this study. A source of error is the settling of cells in the pipette, and this we found greater

in the pipettes with a long tip below the lower graduation, and in bloods diluted with saline instead of serum. On the whole the differences between the B and L Newcomer and the van Slyke in Chart 1 do not show any constant difference between the B and L Newcomer readings and the actual hemoglobin content, but rather the errors inevitable under ordinary working conditions, which are of wider range than we like to believe exist. Comparing our readings with those of Lindsay, Rice and Selinger³ also given on Chart 1, we find their five readings total 638 for the van Slyke against 61 for the Newcomer, ours (sixteen) are 128.4 and 125.9 respectively so close an agree

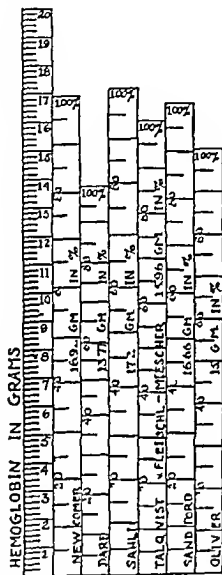


Chart 3—Comparison of arbitrary standards of various hemoglobinometers with accompanying hemoglobin scale for conversion of percentage values

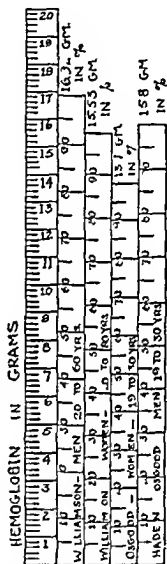


Chart 4—Normal adult hemoglobin standards with scale for conversion from grams to percentage or vice versa

ment that we think with Senter⁴ that the Newcomer (B and L) readings are so similar to the van Slyke that the Newcomer method is dependable, accurate and "of great practical value in eliminating the preparation of standard solutions," provided the B and L disks are used and provided it is recognized that there is a possible irregularity in individual determinations. The Klett disk was consistently too high in readings above about 9 gm per cent which would seem to indicate that the dark glass and 1-400 blood solution are wrong colorimetrically.

While the Newcomer hemoglobinometer is thus accurate for general clinical use, it must be remembered that it suffers from occasional erroneous

readings, a fault with many colorimetric methods, and is not dependable as a research method. Of these latter, all of which must first be standardized by the van Slyke or a ferrometric method, the Palmer method and that of Osgood and Haskins will doubtless give way to the photoelectric hemoglobinometer of Sanford and Sheard⁵ which together with its accuracy has the great advantage of freedom from individual error. The Newcomer disk should be obtained from a firm guaranteeing its accuracy, unless standardization is to be undertaken in the laboratory. Readings with the Newcomer in cases in which the color index is important should be an average of ten readings from each of two blood dilutions. It is generally agreed that hemoglobin should be reported in grams per 100 c.c. Chart 3 is a convenient conversion table from the arbitrary standards of various instruments. It also gives the 16.66 gm standard recommended by Sanford as convenient, in that 6 times the hemoglobin gives the percentage.

Williamson⁶ using a spectroscopic method, measured the hemoglobin of 919 people, about 15 of each sex for each age period, for ages from infancy to extreme old age, thus establishing normals that cannot be affected except as other groups equally large, and as carefully measured, might show that for other regions, races or social conditions other normals hold true. His statistics show a normal of 16.92 gm hemoglobin percentage for men from twenty to sixty years, and 15.53 for women from twenty to eighty years. Since then Haden,⁷ Osgood⁸ and Wintrobe and Miller⁹ have all found the hemoglobin for men from nineteen to thirty years to be about 15.8 gm, and Osgood¹⁰ found an average of 13.7 gm hemoglobin in 100 women of the same age group. As these averages for men and women from nineteen to thirty are from much larger numbers than Williamson's, they are necessarily correct for the student class. In Chart 4 there are both of Williamson's normals for adults, and these later student averages are divided so that a given hemoglobin may be read as percentage of normal for the sex of a patient who is within the given age limits. The normal variation from average should be remembered. Osgood found it from 14 to 18 gm for men (average 15.8), and from 12 to 15.5 (average 13.7) for young women, and Williamson states there is a 10 per cent variation from the average at birth, and 15 per cent in adult life.

CONSTANTS

Hemoglobin 96 per cent globulin, 4 per cent hematin. Iron, 0.334 per cent or 1 mg iron per 300 mg hemoglobin. Oxygen capacity, 1.34 c.c. per 1 gm hemoglobin.

CONCLUSIONS

- 1 Normal hemoglobin means normal for age and sex, and should be determined by Williamson's or other standards, and so recorded.
- 2 The Dare hemoglobinometer should be discarded.
- 3 For clinical work hemoglobin may be estimated by the following methods: (a) Rough bedside estimations may be made by the Tallqvist or the Sahli. If the blood is below normal, a more accurate method should be used.

(b) The Newcomer disk, or the Newcomer hemoglobinometer as furnished by Bausch and Lomb is sufficiently accurate for clinical work. Blood dilutions made with their pipette can be carried to be read at leisure. When the color index is diagnostic, duplicate tests should be made, and an average of ten readings be taken from each dilution. If there is any doubt as to the accuracy of a disk, it should be checked by the van Slyke oxygen capacity method or an accurate ferrometric analysis through a wide range.

4. In the case of adults there is so long a period during which the normal hemoglobin scarcely varies that the percentage of variation may be added as a simple and vivid comparison, along with the grams per 100 c.c. blood. For infants and children the percentage of an arbitrary male standard, as used in the past, is only misleading. The hemoglobin observed should be recorded in grams together with the normal for age and sex, which can be interpolated from Williamson's curve.¹¹

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COLORIMETRIC ESTIMATION OF THE URINARY P_H *

BY VICTOR C MYERS, AND EDWARD MUNTWYLER, CLEVELAND, OHIO

AS ORDINARILY carried out the colorimetric estimation of the hydrogen-ion concentration of urine gives results which are too high by approximately 0.2 P_H , if one desires to know the P_H of the undiluted urine at body temperature. While it is true that an error† of 0.2 P_H in the estimation of the P_H of urine is of relatively little significance owing to the wide variations which may be encountered normally (4.8-8.0 P_H), still one should always know the accuracy and limitations of the method employed. In the past it has been assumed that a 10 or 25 fold dilution was without influence on the P_H of urine, and these dilutions are the ones which have been employed for urine. Hastings and Sendroy¹ have suggested a 1 to 5 dilution with water, while still more recently Myers and Muntwyler² have employed this dilution, but used as a diluent a saline solution because of its greater stabilizing effect on the dilution error. Employing water the error of dilution may be extremely variable. Diluting a urine of very high P_H where the carbonates form the major buffers will show a much greater dilution error than a highly concentrated urine of low P_H where phosphates form the buffers. By employing saline solutions with the reaction adjusted to suit each indicator range, this variable affect of water dilution is minimized and consequently makes all results comparable.

These same principles probably apply to fluid bacteriologic culture media as well as to urine, since one should have essentially the same temperature and dilution errors. Of course if a plus error as great as 0.3 P_H is of no consequence, then these errors need not be considered.

For their work Myers and Muntwyler² employed the bicolorimeter³. Owing to the fact that this instrument is not available in all laboratories it has seemed helpful to redescribe this method for use with the comparator box.

During the past five years we have employed the comparator box,† illustrated in Fig. 1, for class work and found it very satisfactory. As will be noted the cover and the bottom part of the box are in reality two comparator boxes and may be used as such when the urine is perfectly clear, and it is not necessary to employ a double row of tubes to obtain a satisfactory match. When not in use the cover is kept on the comparator box so that the buffer-indicator solutions will not be exposed to light.

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†Although the object of the present paper is to point out sources of error in the customary estimation of the urinary P_H and describe conditions which permit its estimation with an error of less than 0.1 P_H , still there are conditions under which this accuracy is unnecessary. The La Motte Chemical Products Company list a Urinary P_H Outfit in which, with the aid of a duplex indicator (combination of two indicators) the range P_H 4.4 to 8.0 is covered by 10 tubes differing by 0.4 P_H . This should be quite satisfactory for ordinary clinical work. They also make a similar comparator box containing 19 tubes differing by 0.2 P_H which covers the P_H range 4.0 to 8.0.

‡This special comparator box may be obtained from the La Motte Chemical Products Company, Baltimore, Md.

The Clark Labs series of indicators are admirably adapted to the determination of the P_H of urine. The indicators bromeresol green (P_H 3.8 to 5.4), bromeresol purple* (P_H 5.2 to 6.8), bromthymol blue (P_H 6.0 to 7.6), and phenol red (P_H 6.8 to 8.4) nicely cover the range of hydrogen ion concentration which may be encountered in urine. For most urines it will be found unnecessary to use the indicators bromeresol green and phenol red, although with urines which are strongly acid or those which are alkaline these two indicators may be necessary. Most urine specimens fall within the range of the indicator, bromeresol purple.

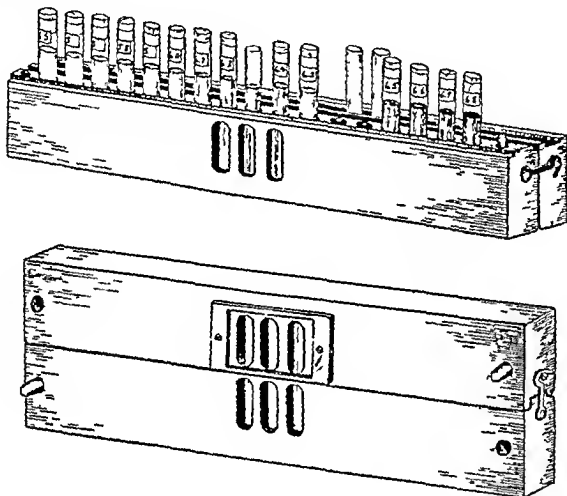


Fig. 1

A series of Sørensen's M/15 molecular buffer phosphates are employed for the standards from P_H 5.2 to 8.6. For the range from 4.4 to 5.2 Clark's phthalate NaOH standards are employed. The different standards are made up to differ by P_H 0.1, and it is therefore possible by interpolation to read to P_H 0.05.

INDICATOR DILUTING SOLUTIONS

The indicator diluting solutions are prepared as follows. To 780 cc. of 0.9 per cent sodium chloride solution 100 cc. of the desired diluted indicator solution are added. This when diluted 4 to 5, gives the same concentration of dye as in the standard. Bromeresol purple, bromthymol blue, and bromeresol green in 0.04 per cent strength and phenol red in 0.02 per cent strength are prepared from 0.4 per cent stock solutions by dilutions of 1 to 10 or 1 to 20 with distilled water.

Chlorphenol red covers the same P_H range as bromeresol purple and may be substituted for it. The former has the advantage that it does not show dichromatism and is more stable in the presence of strong alkali than bromeresol purple.

A SIMPLIFIED METHOD FOR WHITE BLOOD CELL COUNTS THAT IS APPLICABLE TO FIELD CONDITIONS¹

BY H A HOFFMAN, D V M, ZIONSVILLE, IND

THE generally accepted methods of collecting blood for either the red or the white cell count are not practicable in the field unless the clinician carries the microscope to the patient. The difficulties attendant upon this procedure seriously limit the use of blood study as an aid in diagnosis.

Lewis and Shope¹ in studying blood from swine, suggest the use of potassium oxalate in undiluted blood to prevent clotting while the latter is being transported to the laboratory. I found that crystals of potassium oxalate appeared upon the counting chamber and interfered with the count. Blood which is treated with potassium oxalate must be diluted within a few hours to avoid spoilage by bacterial contamination.

The present study was undertaken in an effort to develop a technique whereby clinicians could conveniently collect samples and send them to a laboratory for the counts. Pigs' blood was used throughout the experiment.

An effort was made to dilute the blood immediately after bleeding and to eliminate the use of the standard diluting pipette.

Technic

Nine and five tenths c.c. of diluting fluid were measured accurately into 6 inch test tubes that were equipped with rubber stoppers and a few glass beads.

The animals were bled from one of the ear veins with an 18 gauge needle $3\frac{1}{4}$ inches in length. Approximately one cubic centimeter of blood was collected into a second test tube and by aid of a graduated 1 c.c. pipette exactly 0.5 c.c. of blood were measured into the tube containing the diluting fluid. This gave a dilution of one part of blood in nineteen parts of diluent. Care should be exercised to wipe the excess blood from the surface of the pipette.

In making the counts the tubes of diluted blood were vigorously shaken for exactly thirty seconds. The cover glass was placed upon the counting chamber and the diluted blood was transferred from the test tube to the counting chamber by means of a capillary pipette prepared from ordinary glass tubing. The blood was allowed to flow underneath the cover glass by capillary attraction. In an effort to check the technique of shaking the diluted blood, each half of the counting chamber was prepared and counted separately. The dilution tube was shaken exactly thirty seconds before transferring the blood to the counting chamber in each case.

All preparations were allowed to stand five minutes before making the counts. In making the count the technique recommended by Piney² was used.

Early in the work it was found that the diluting fluids recommended by Piney,³ Mallory and Wright,⁴ Cummei,⁵ or Burnett,⁷ which consisted of various dilutions of acetic acid tinged with carbo gentian violet, were not adequate. If the counts were made within a few hours very little difficulty was experienced, but, if the counts were made some time after making the dilutions, the micro-

¹From the Research Department Allied Laboratories Inc.
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scopic field was often clouded with debris and the cells were swollen, distorted or indistinct

Twenty four different preparations of various acids were tried. A description of the more promising results together with the outstanding failures are reported in Table I. Two stains were used in these tests. They consisted of a

TABLE I
SUMMARY OF RESULTS WITH VARIOUS DILUTING FLUIDS

CODE NUMBER	COMPOSITION OF DILUTING FLUID	AGE OF DILUTED BLOOD WHEN COUNTED	TEMPERATURE OF STORING DILUTED BLOOD	OBSERVATIONS IN COUNTING
1 A	2% acetic acid faintly tinged with carbo gentian violet	few hours	room temp	The field was not clear clumps of red cells and other debris interfered with the count
3 A	2% acetic acid with 1% carbo gentian violet	5 days	room temp	Red cells faintly visible white cells slightly brown not swollen
5 A	1% acetic acid with 0.5% carbo gentian violet	few hours	room temp	Clear field white cells distinct
7 A	2% acetic acid with 1% methyl violet solution	5 days few hours 3 days	5 C room temp room temp	Cells not clear difficult to locate Cells slightly stained quite distinct Cells slightly stained quite distinct Very little debris
10 A	3% lactic acid† with 1% methyl violet solution	few hours 1 day	room temp room temp	Cells slightly stained quite distinct, some debris Cells slightly stained some debris
11 A	2% citric acid with 1% methyl violet solution	few hours 1 day	room temp room temp	Cells slightly stained, quite distinct some debris Cells overstained very little debris
12 A	2% oxalic acid with 1% methyl violet solution	few hours 1 day	room temp room temp	Cells a brownish tint contrast to color of debris Cells quite distinct Field covered with small and large particles Cells yellowish brown
13 A	4% oxalic acid with 1% carbo gentian violet	few hours 1 day	room temp 5 C	Cells sharply outlined morphology distinct stained lemon yellow very little debris Cells a bright yellow morphology quite distinct cells not distorted A very fine debris over the field but does not interfere with count
20 A	5% oxalic acid with 1% methyl violet solution	few hours	room temp	Cells clear and distinct stained a yellow color
16 A	3% citric acid with 1% methyl violet solution	few hours 1 day	room temp 5 C	Some debris of red cells with cells stained faint lavender cytoplasm clear but visible nuclei stained cells stained but not clearly much debris

The acetic acid used was Merck 30 per cent

†The lactic acid was 10 per cent strength

standard preparation of carbo gentian violet⁵ and a 1 per cent aqueous solution of methyl violet. When methyl violet was used in the diluting fluids the amount was 1 per cent of the aqueous solution.

The most satisfactory preparations were 12 A and 20 A which consisted of 2 per cent and 5 per cent oxalic acid respectively and 1 per cent of a 1 per cent methyl violet solution. The yellow stained cells stood out in relief and were quickly and easily seen. The preparation 13 A, which contained 3 per cent

oxalic acid and 1 per cent carbo-gentian violet was almost as satisfactory. These three preparations were much superior to any of the others tried in the experiment.

Comparisons were made between the standard method of collecting blood samples using the hemacytometer, and the test tube method described in this article. Ten such comparisons were made by taking duplicate samples of blood from the same animal at the same time. The hemacytometers were filled directly from the hypodermic needles, while the blood for the tube tests was collected in separate test tubes. The average of the counts for these tests by the hemacytometer method was 13423 while the average by the test tube method was 12554.

An effort was then made to definitely check the error of both methods by making a series of counts from the same animal at the same bleeding. February 27 1929, Pig No. 3055 was bled from the ear with a hypodermic needle. Five separate hemacytometers were filled one after the other. Five dilutions were then made from same needle into test tubes containing dilution fluid. The diluting fluid used was No. 20 A (5 per cent oxalic acid with 1 per cent of a 1 per cent aqueous solution of methyl violet). The dilution in each test was one in twenty.

The counts were made in duplicate using separate counting chambers. Each dilution was shaken exactly thirty seconds before transferring to the counting chamber. The cover glasses were placed upon the slides and the diluted blood was introduced from the edge. Two drops of diluted blood were discarded from each pipette. The third was wiped off with a clean cloth and the fourth was placed upon the slide. In each instance the preparation was allowed to stand five minutes before making the count.

TABLE III
HEMACYTOMETER METHOD

TUBE NO	FIRST COUNT	SECOND COUNT	AVERAGE OF FIRST AND SECOND	DIFFERENCE OF FIRST AND SECOND
143	4960	3960	4460	1000
144	4200	4520	4360	320
145	5120	2920	4020	2300
146	5040	4480	4760	560
147	5960	5200	5580	760
Averages	5056	4216	4636	988
Range of difference by the hemacytometer method 3040				

TABLE IV
TEST TUBE METHOD

TUBE NO	FIRST COUNT	SECOND COUNT	AVERAGE OF FIRST AND SECOND	DIFFERENCE OF FIRST AND SECOND
148	4200	4640	4420	440
149	4480	4400	4440	80
150	4040	4560	4300	520
151	4600	4560	4580	40
152	4280	5080	4630	800
Averages	4212	4648	4470	376
Range of difference by the test tube method 1040				

Table III records the counts by the hemacytometer method and Table IV records the counts by the test tube method. All of the counts represent the blood from the same animal at the same bleeding.

The average count by the hemacytometer method was 166 higher than by the test tube method. With the former method the range of differences was 2000 greater than by the test tube method.

The tubes numbered 148 to 152 inclusive were set aside at room temperature for five days.

On March 4 these tubes were recounted with the following results:

TUBE NO	FIRST COUNT	SECOND COUNT	AVERAGE COUNT	DIFFERENCE BETWEEN TWO COUNTS
148	5080	4920	5000	160
149	4180	4400	4440	80
150	4600	4840	4720	240
151	4920	3920	4420	1000
152	3920	4560	4290	640
Averages	4600	4528	4574	424
Range of differences 1160				

DISCUSSION

By making use of a previously measured tube of diluting fluid and a graduated pipette dilutions of blood may be transported to the laboratory without danger of loss of the specimen. This is not possible when the standard diluting pipette is used. Subsequent to these experiments a few tests were made in which the amount of diluting fluid was reduced to 19 cc and the amount of blood to 0.1 cc. In these tests the blood was collected from a drop from a needle puncture or directly from the needle. The results indicate that the latter technique might well be substituted for the larger amounts of blood and diluting fluid.

When proper care is exercised in making the dilutions and in preparing the slide for the count, the results are more uniform when a pipette and a test tube are used in making the dilutions. The uniformity of count depends to a marked degree upon adequate shaking of the diluted blood. This technique enables the worker to transport the sample without danger. The count can be made at his convenience.

A more satisfactory preparation can be obtained by the use of 2 per cent to 5 per cent oxalic solution with 1 per cent of a 1 per cent methyl violet as a diluting fluid.

I wish to acknowledge the suggestion from Doctor S. H. Regenes to use a test tube in making the dilutions of blood.

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BLOOD SUGAR METHODS FROM A CLINICAL POINT OF VIEW*

BY O B PRATT, M D , AND H O SWARTOUT, B A , M S , LOS ANGELES, CALIF

SINCE 1920, when Folin and Wu¹ announced their improved procedure, now commonly known as the "standard Folin-Wu blood sugar method," many workers have tested it as to its chemical accuracy and clinical usefulness under varying conditions. Though it is probably still more widely used than any other blood sugar method, at times changes in technique have been proposed, and other methods have been suggested as superior to it. Most of the reports of work along these lines have appeared in the *Journal of Biological Chemistry*. Naturally, therefore, the chemical side of blood sugar methods has been emphasized more than the clinical.

During recent years the clinical importance of blood sugar tests has greatly increased, due largely to insulin therapy. A reasonably accurate idea of a patient's blood sugar level is often a prime point in diagnosis and a valuable guide in treatment. Biochemists are properly interested in the correct figure for average normal blood sugars, and in the question as to whether what appears to be blood sugar is really all sugar or partly one or more other compounds, but such matters are of less clinical moment. The clinician's purpose is adequately served, and the work of the clinical laboratory is made useful and dependable by any blood sugar method which, in the hands of the ordinary technician, gives a good check on the amount of deviation from normal in the case of both high and low sugars, no matter what the normal level for that particular method may be.

The work of several investigators who have attacked the blood sugar problem from the chemical side has clinical aspects which we believe deserve the review we aim to give them in this paper. The results of some of our own recent tests will also be considered, as they have a bearing on the subject. Of necessity, part of the discussion must concern itself with the chemistry of blood sugar methods, but more emphasis will be put upon those features which determine their clinical usefulness.

In 1923 Rothberg and Evans² announced the results of a series of studies of the standard Folin-Wu method. They found that it gave results too high for high sugars and too low for low sugars. In 1926 Oser and Kari³ reported tests indicating similar errors, though they found the deviations somewhat less than those noted by Rothberg and Evans. Rockwood⁴ confirmed the findings of Oser and Kari in this respect, and the results of our tests are in general agreement with theirs. Our figures indicate that, when using the single standard, a blood sugar at a level of about 200 mg per 100 cc of blood will give results from 10 per cent to 12 per cent too high, while one near the 50 mg

*From the Research Laboratory of the White Memorial Hospital, College of Medical Evangelists.

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level will turn out from 15 per cent to 18 per cent less than its true value—differences too great to be disregarded by the careful clinician, especially in the case of the low sugars

Rothberg and Evans attributed these deviations to the abnormal behavior of colored colloidal solutions when diluted to different degrees. They advised the use of several different standards, together with a special type of tube which would permit of adjusting the dilutions, as a means of overcoming the difficulty. Our check tests only partly confirmed their conclusions. But granting that their work was free from error the added details of technique which they suggested would make the method of analysis unsatisfactorily complex for routine use.

For the purpose of calculating the proper values for blood sugars from observed colorimeter readings, Oser and Kari advised the use of a special table, corrected so as to offset the deviations indicated by their tests. This is a practical solution of the problem, and in our routine work we for a considerable time used such a table. Observation and inquiry, however, led us to the opinion that most laboratories employing the Folin Wu method do not use corrected tables. As a consequence, much too low reports on low blood sugars must be very common.

Much of the blood sugar work during the past three or four years has been aimed at perfecting reagents or methods that would be specific for glucose as distinguished from other reducing substances in the blood. While of great chemical interest not much of this work is an improvement on the standard Folin Wu method from a clinical viewpoint. We may at once rule out as ill adapted for practical laboratory purposes all the methods which include yeast fermentation as a step in technique. These require the attention of a worker of more than average competence, as well as carefully controlled conditions, or else serious errors will often be made. Van Slyke and Hawkins¹⁵ gasometric method is cumbersome and time consuming, compared with the Folin Wu method. A new copper reagent proposed by Folin⁶ early in 1926 is so weakly alkaline that all of the blood filtrates must for safety be neutralized to phenolphthalein before testing—a fact that argues against the routine use of this reagent. Furthermore later in the same year Folin and Svedberg⁷ in giving directions for handling the new copper reagent brought to light another fact which makes it objectionable, it is not stable unless it is kept in full, tightly stoppered bottles until shortly before using. Benedict's⁸ 1928 copper reagent gives results that are believed to be very nearly correct, but it is also open to the criticism of instability and inconvenience. He advised that this reagent be kept as two separate solutions which should be mixed not more than a day or two before being used. His⁹ 1926 reagent is better, both as to convenience and keeping qualities.

At this point we digress briefly to note some interesting items connected with our own work. We have made hundreds of tests of the standard Folin Wu method, as applied both to aqueous solutions of glucose and to blood filtrates with suitably adjusted glucose content, the tests covering the range corresponding to from 50 mg to 400 mg of glucose per 100 cc of blood. Our original purpose was to obtain data for a corrected table, such as proposed by Oser and

1000 PRECIPITATION TESTS FOR SYPHILIS WITH SMALL QUANTITIES OF DEFIBRINATED FINGER BLOOD (CLINICAL AND SEROLOGIC COMPARISON)*

By B S KLINE, M D , AND BENJAMIN LEVINE, M D , CLEVELAND, OHIO

THE simple precipitation test for syphilis with small quantities of defibrinated finger blood described previously¹ has been found in 1000 tests to be more sensitive than the serum Wassermann test with the same antigen and almost as sensitive as the serum microscopie slide precipitation test. No false positive reactions occurred in any of the tests in this series. In 121 cases, the finger blood test performed with the slight changes noted below gave results agreeing with the clinical condition of the patients as often as those of the serum microscopie slide precipitation test and more frequently than those of the serum Wassermann test.

The finger blood precipitation test for syphilis is satisfactory for use in the diagnosis of syphilis in all cases and since it requires but a small quantity of comparatively easily obtainable blood, it is particularly useful in the diagnosis of syphilis in infants and children. Furthermore, after satisfactory blood typing and matching the finger blood test for syphilis, since it is reliable and takes but about eight minutes to do, is an excellent method for determining the suitability of blood donors immediately before transfusion.

For the study presented below, blood was obtained from the finger and defibrinated for the finger blood test. At the same time a larger quantity was drawn from an arm vein to furnish serum for the microscopie slide precipitation test and for the Wassermann test. Over 60 per cent of the blood specimens were obtained from syphilitic patients. Of these over 80 per cent showed \pm to ++++ reactions in one or more tests.

The clinical and serologic comparison of the finger blood test with other tests for syphilis is given in Tables I to IV.

Table I shows the close agreement of the finger blood test with the serum slide precipitation test. There is less agreement of the finger blood test with the Wassermann test.

Table II shows the results of the finger blood test to be more sensitive than those of the Wassermann test and almost as sensitive as those of the slide precipitation test.

Table III shows the false negative reactions in the various stages of syphilis. The very sensitive finger blood test gave one and a half times as many false negative reactions as the very sensitive microscopie slide precipitation test, whereas the very sensitive Wassermann test gave over two and one-half times as many.

*From the Laboratory Department and the Department of Syphilology of the Out-patient Clinic Mount Sinai Hospital.
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level will turn out from 15 per cent to 18 per cent less than its true value—differences too great to be disregarded by the careful clinician, especially in the case of the low sugars.

Rothberg and Evans attributed these deviations to the abnormal behavior of colored colloidal solutions when diluted to different degrees. They advised the use of several different standards together with a special type of tube which would permit of adjusting the dilutions as a means of overcoming the difficulty. Our check tests only partly confirmed their conclusions. But, granting that their work was free from error the added details of technique which they suggested would make the method of analysis unsatisfactorily complex for routine use.

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Kair We give an abridged report of average results below, however, so that a rather surprising feature, noted after the tests were completed, may more easily be demonstrated

ACTUAL CONCENTRATION OF GLUCOSE IN TEST SOLUTIONS	CALCULATED CONCENTRATION OF GLUCOSE BY FOLIN WU TESTS	ACTUAL CONCENTRATION OF GLUCOSE IN TEST SOLUTIONS	CALCULATED CONCENTRATION OF GLUCOSE BY FOLIN WU TESTS
mg	mg	mg	mg
50	42	100	100
60	54	120	123
70	65	140	147
80	77	160	170
90	89	180	195

While studying our results it occurred to us that if a quantity of color equivalent to 15 mg or 16 mg of glucose per 100 cc of blood could be added to each tube, standard included, the whole series would be brought so nearly into line that no result would differ much from what it ought to be. For instance, the tube which, compared with a standard of 100 mg per 100 cc gave a reading that calculated out at 42 mg per 100 cc should have given 50 mg per 100 cc, that which led to a report of 170 mg per 100 cc should have given 160 mg per 100 cc, etc. Now the ratio of $(42 + 15) (100 + 15)$ nearly equals 50 100, $(42 + 16) (100 + 16)$ equals 50 100, $(77 + 15) (100 + 15)$ equals 80 100, $(77 + 16) (100 + 16)$ nearly equals 80 100, $(170 + 15) (100 + 15)$ nearly equals 160 100, $(170 + 16) (100 + 16)$ nearly equals 160 100, and so on through the list.

The above figures were derived from tests made with single standards, but we found the same mathematical relations to hold in the case of results obtained by using more concentrated test solutions read against double standards. We make no pretense of knowing all the reasons for this peculiarity. It may be that the deviation of colored colloidal solutions from Beer's law²⁰ partly explains it. Perhaps something in the copper reagent affects the glucose in such a way as to prevent a fairly constant quantity of it in each tube from entering into the reduction reaction. The fact that some tests which we made on solutions representing 20 mg of glucose per 100 cc of blood gave almost no color at all points to this possibility. But no matter what the explanation may be, it is evident that if the copper reagent of Folin and Wu could be modified so as to aid in the production of added color to the extent called for by our figures, their method would give proportionately accurate results for both high and low sugars.

With this thought in mind we set out to alter the Folin-Wu copper reagent in the desired direction, meanwhile reinvestigating such other reagents as were not clinically undesirable from other points of view. We had already met with an encouraging measure of success when we began testing Benedict's 1926 copper and acid reagents. These gave us results so nearly correct that we went no further with our work in modifying the Folin-Wu reagent, feeling that it would serve no good purpose to develop a new reagent that would be no improvement over one already proposed.

According to our tests these Benedict reagents, used with standard Folin Wu filtrates, lead to reports on blood sugars as low as 40 mg per 100 cc that in proportion to each other and to the standards employed are accurate within the limits of observational error. On high sugars the error may run as high as 2 per cent or 3 per cent, the reports being too low to this extent. Such a deviation in the case of a high sugar is clinically insignificant, but if desired it can easily be avoided by using a smaller quantity of filtrate for the test.

It may be of interest to mention that when testing Benedict's 1926 reagents we did not find the blanks negligible. Since, however, the slight color due to the blanks affects the standards as well as all of the test solutions, we have here a possible partial explanation of the closer agreement between actual and theoretical sugar values which these reagents give as compared with the standard reagents of Folin and Wu. It is more color in all the tubes which is needed to bring the Folin Wu results into line, and the blanks of Benedict's reagents supply a little additional color.

We have found Benedict's 1926 copper reagent to have enough alkalinity to offset the acidity of filtrates from blood samples that have been treated with many times the recommended quantities of any of the common anticoagulants, comparing favorably with the standard Folin Wu reagent in this respect. This is a valuable feature in practical work for the average clinical laboratory must often deal with blood samples which have been treated with a considerable excess of the compounds added to prevent clotting.

CONCLUSION

While Benedict's 1928 copper reagent gives results that are probably nearer the correct levels for blood sugars, his 1926 reagent is preferable from the standpoints of stability and convenience. Though this reagent may lead to results that are slightly too high all along the line, these results are consistent among themselves and closely proportional to the standards. Our tests indicate that Folin Wu blood filtrates used with Benedict's 1926 copper and acid reagents made according to directions given in the *Journal of Biological Chemistry*¹¹ provide a blood sugar method that is suited to the ordinary clinical laboratory and that will give results upon which the clinician can safely base his diagnoses and treatments. We know of one large hospital that is using this combined method at present. There may be others. We feel that we are justified in adopting it as a routine procedure in our own laboratory.

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A CHART FOR ADJUSTING THE DIET IN DIABETES*

By CURTIS BRUEN, M D, NEW YORK CITY

NITROGENOUS equilibrium, caloric requirement, ketogenic-antiketogenic balance, and glucose equivalent tolerance together determine the diabetic diet. The wear and tear quota fixes the protein minimum. The caloric requirement is satisfied according to the equation,

$$C = 4.1CH + 4.1P + 9.3F \quad (1)$$

Ketogenic antiketogenic balance is maintained by the relationships of the equation,

$$F = 2CH + 0.546P \quad (2)^1$$

Tolerance sets a limit within which the glucose equivalent must fall unless insulin be given.

Charts are available for reading off food mixtures in ketogenic-antiketogenic balance. The alignment chart of Wilder based on a special gram molecular calculation indicates the grams of carbohydrate and fat required to meet the caloric requirement with a given protein allowance without clinically significant accumulation of acetone bodies². Given the total calories and the percentage of the total calories to be furnished by protein, the complex line diagram of Hammon and McCann derived from the above equations³ indicates the grams of protein, the grams of carbohydrate, and the factor by which the grams of carbohydrate is multiplied to give the grams of fat⁴. A single diet follows from each set of conditions.

Graphs of simultaneous equations permit of more flexibility. A system of graphs with serial protein values from a near-minimal wear and tear quota⁵ to an arbitrary upper limit is drawn for equation (2). The segments above their intersections with the individual graphs of this system are drawn for systems of graphs for equation (1) having corresponding protein values but serial values of total calories. The widest range of practicable values of the several food-stuffs which satisfy the equations individually and simultaneously is provided.

Chart I Diabetic Diet—Directions: (1) Identify the graph for the required number of grams of protein, (2) run along it to the point where a member of the bank of graphs for the required number of calories rises from it, (3) read off the coordinates of this point for the number of grams of carbohydrate and fat in the threshold diet, (4) read off the coordinates of points higher up on this graph for diets of increasing carbohydrate, (5) read off the coordinates of points along its imaginary projection beyond the intersection for diets of impending ketosis.

A suitable combination is selected from among the serial values of protein and of calories available. The indicated graph of caloric equivalent mix-

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tures of carbohydrate and fat satisfies these conditions throughout its length. Its point of simultaneity with the graph of ketogenic antiketogenic balance satisfies in addition the relationships of the threshold of ketosis. Values within this limit are more remote from the development of ketosis but require more of the capacity to metabolize glucose. Values beyond it require less of

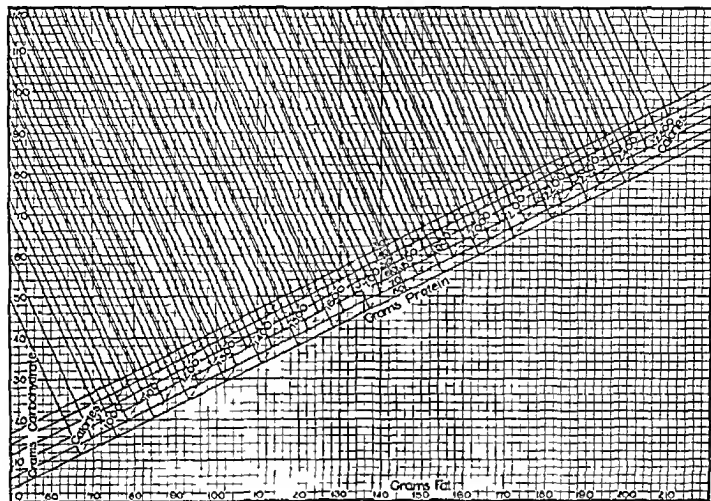


Chart 1

the capacity to metabolize glucose but are less proof against the development of ketosis. The diet can be maintained at once along physiologic lines and according to the clinical indications.

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A GLASS LIGHT FILTER FOR FOLIN'S NEW MICRO BLOOD SUGAR METHOD*

By HENRY TAUBER, PH D, BROOKLYN, N Y

FOLIN, in one of his later papers,¹ suggests the use of a colored filter paper or a special lamp as a light filter to get uniformity in reading because of the disturbing effect of the excess of the yellow potassium ferricyanide solution

We have cooperated with the Klett Manufacturing Co in the preparation of a glass light color filter which is a yellow glass disc, 15 mm in diameter, which should be placed on top of the ocular of the colorimeter

TEST OF THE GLASS LIGHT COLOR FILTER

The glass color filter is placed on the ocular of the colorimeter One cup is filled half with a 0.2 per cent potassium ferricyanide solution and the other cup is filled half with water Both plungers are set at 20 mm and, if zero point and light are equal, the two fields look uniformly yellow

Table I shows comparative figures of Folin's micro method with the glass disc color filter and the original Folin-Wu method The bloods are those of diabetic patients

TABLE I

SAMPLE NO	SUGAR IN MG PER CENT		
	FOLIN WU	FOLIN	DIFFERENCE
1	222	220	2
2	160	148	12
3	320	312	8
4	142	135	7
5	266	252	14
6	400	388	12
7	166	156	10
8	160	153	7
9	117	111	6
10	306	290	16
11	250	228	22
12	220	217	3
13	152	147	5
14	302	290	12
15	92	86	6
16	105	100	5

This corroborates Folin's² lower findings with the micro method

Table II shows figures of glucose solutions of various concentrations

It is important to note that the lower findings of the micro method as compared to Folin-Wu's original method are due to the method itself as seen by the fact that the figures in both the glucose solutions and blood sugars are lower

*From the Department of Laboratories of Beth Moses Hospital Brooklyn N Y
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TABLE II

SOLUTION NO	MG PER CENT GLUCOSE	FOLIN	DIFFERENCE
1	150	148	2
2	200	106	4
3	250	235	15
4	300	200	10
5	350	345	5
6	400	392	8
7	450	445	5
8	500	482	18
9	550	533	17
10	600	500	10
11	700	660	40

For the precipitation of the blood proteins in Folin Wu's method, we used a precipitation mixture consisting of 7 parts of distilled water, 1 part of a 10 per cent sodium tungstate solution, and 1 part of 2/3 N sulphuric acid. To 9 parts of this mixture in an Erlenmeyer flask, 1 part of blood is added, drop by drop, while rotating the flask, and filtered after a few minutes.

We have made a great number of blood sugar determinations using this mixture, which we compared with the original method of precipitation as used by Folin and Wu, and found that both results agree. Occasionally, a precipitate is formed in the mixture which has no effect on the blood sugar values.

The advantages of the glass disc as compared to Folin's filter paper light screen or special lamp are

- 1 The disc saves time in routine examination
- 2 The filter light papers tend to curl up due to the heat of the colorimeter lamp
- 3 The disc is inexpensive and can be used for any plunger

The author is indebted to Mr Klett and Mr Daniel for their assistance in preparing the disc.

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1000 PRECIPITATION TESTS FOR SYPHILIS WITH SMALL QUANTITIES OF DEFIBRINATED FINGER BLOOD (CLINICAL AND SEROLOGIC COMPARISON)*

By B. S. KLINE, M.D., and BENJAMIN LEVINE, M.D., CLEVELAND, OHIO

THE simple precipitation test for syphilis with small quantities of defibrinated finger blood described previously¹ has been found in 1000 tests to be more sensitive than the serum Wassermann test with the same antigen and almost as sensitive as the serum microscopic slide precipitation test. No false positive reactions occurred in any of the tests in this series. In 121 cases, the finger blood test performed with the slight changes noted below gave results agreeing with the clinical condition of the patients as often as those of the serum microscopic slide precipitation test and more frequently than those of the serum Wassermann test.

The finger blood precipitation test for syphilis is satisfactory for use in the diagnosis of syphilis in all cases and since it requires but a small quantity of comparatively easily obtainable blood, it is particularly useful in the diagnosis of syphilis in infants and children. Furthermore, after satisfactory blood typing and matching, the finger blood test for syphilis, since it is reliable and takes but about eight minutes to do, is an excellent method for determining the suitability of blood donors immediately before transfusion.

For the study presented below, blood was obtained from the finger and defibrinated for the finger blood test. At the same time a larger quantity was drawn from an arm vein to furnish serum for the microscopic slide precipitation test and for the Wassermann test. Over 60 per cent of the blood specimens were obtained from syphilitic patients. Of these over 80 per cent showed \pm to ++++ reactions in one or more tests.

The clinical and serologic comparison of the finger blood test with other tests for syphilis is given in Tables I to IV.

Table I shows the close agreement of the finger blood test with the serum slide precipitation test. There is less agreement of the finger blood test with the Wassermann test.

Table II shows the results of the finger blood test to be more sensitive than those of the Wassermann test and almost as sensitive as those of the slide precipitation test.

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*From the Laboratory Department and the Department of Syphilology of the Out-patient Clinic, Mount Sinai Hospital.

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TABLE II
CLINICAL COMPARISON OF FINGER BLOOD TEST AND SERUM TESTS FOR SYPHILIS WITH THE SAME ANTIGEN

	FINGER BLOOD PRECIPITATION TEST				HEATED SERUM SLIDE PRECIPITATION TEST				HEATED SERUM WASSERMANN TEST			
	VERY SENSITIVE EMULSION		SENSITIVE EMULSION		VERY SENSITIVE EMULSION		SENSITIVE EMULSION		VERY SENSITIVE EMULSION		SENSITIVE EMULSION	
	AGREE MENT	DISAGREE MENT	AGREE MENT	DISAGREE MENT	AGREE MENT	DISAGREE MENT	AGREE MENT	DISAGREE MENT	AGREE MENT	DISAGREE MENT	AGREE MENT	DISAGREE MENT
Syphilitic sera this series ± to +++ in one or more tests	370 87.47%	53 12.53%	397 76.79%	120 23.21%	337 91.49%	36 8.51%	454 87.81%	63 12.19%	319 77.80%	91 22.20%	256 55.29%	207 44.71%
Syphilitic sera this series negative to +++	445 89.36%	53 10.64%	501 80.68%	120 19.32%	462 92.77%	36 7.23%	558 89.86%	63 10.14%	394 81.24%	91 18.76%	350 62.84%	207 37.16%
Syphilitic and nonsyphilitic sera this series (over 50% positive syphilitic sera)	716 93.11%	53 6.89%	871* 87.89%	120† 12.11%	723 95.26%	36 4.74%	928 93.64%	63 6.36%	650 87.72%	91 12.28%	674 76.50%	207 23.50%
Approximate result if series contained 20% positive syphilitic sera (high average)	Approx 2062 97.49%	53 2.51%	Approx 2465 95.36%	120 4.64%	Approx 2079 98.30%	36 1.70%	Approx 2522 97.56%	63 2.44%	Approx 1959 95.56%	91 4.44%	Approx 2108 91.06%	207 8.94%

*871 †120 and 0 = 1000 total tests

Evaluation

Negative in all tests with syphilitic sera or non-syphilitic sera = Agreement

+ to +++ in all tests with syphilitic sera = Agreement

Negative in one test ± to +++ in one or both other tests with syphilitic sera = Disagreement.

0 Clinically doubtful cases as follows

	FINGER BLOOD TEST		SERUM PRECIPITATION TEST		WASSERMANN TEST	
	VERY SENSITIVE		VERY SENSITIVE		VERY SENSITIVE	
	SENSITIVE	VERY SENSITIVE	SENSITIVE	VERY SENSITIVE	SENSITIVE	VERY SENSITIVE
++		1				
+ or +	4	4	2	2		
Negative	5		7	3	9	5
Total cases	9	5	9	5	9	5

TABLE III

CLINICAL COMPARISON OF TESTS FOR SYPHILIS ONE OR MORE OF WHICH GAVE A \pm TO ++++ REACTION WITH ANALYSIS OF DISAGREEMENTS

	TOTAL TESTS	AGREEMENT	DISAGREEMENT	DISAGREEMENTS FALSE NEGATIVES, SYPHILIS				
				PRIMARY	SECONDARY	TERTIARY	CENTRAL NERVOUS SYSTEM	CONGENITAL
Very sensitive finger blood test	423	370 87.47%	53 12.53%	8 1.89%	30 7.09%	7 1.65%	4 0.95%	4 0.95%
Very sensitive slide test	423	387 91.49%	36 8.51%	10 2.36%	16 3.78%	7 1.65%	1 0.24%	2 0.47%
Very sensitive Wassermann test	410	319 77.80%	91 22.20%	14 3.41%	38 9.27%	34 8.29%	1 0.24%	4 0.98%
Sensitive finger blood test	517	397 76.79%	120 23.21%	22 4.26%	66 12.77%	22 4.26%	5 0.97%	5 0.97%
Sensitive slide test	517	454 87.81%	63 12.19%	11 2.13%	34 6.58%	15 2.90%	1 0.19%	2 0.39%
Sensitive Wassermann test	463	256 55.29%	207 44.71%	22 4.75%	111 23.97%	61 13.17%	6 1.30%	7 1.51%

Table IV shows the results of the finger blood test in 121 cases, by the technique described below, to be as sensitive as those of the slide precipitation test and more sensitive than those of the Wassermann test

DISCUSSION OF THE TECHNIC OF THE FINGER BLOOD PRECIPITATION TEST FOR SYPHILIS

Many of the finger blood tests reported above were done as outlined in a previous report¹. In addition, many were done with antigen emulsions of different cholesterol content with, consequently, different optimum quantities of salt solution. Furthermore, it was found that the addition of a small quantity of acetic acid to the sensitive antigen emulsion increased its sensitivity equal to that of heating it at 50° C for twenty minutes. It was also found that more cholesterol could be used in the antigen emulsions with advantage. A most important factor affecting the results was found to be the hydrogen ion concentration of the distilled water used in the preparation of the antigen emulsions and the salt solution. It was also found better to take the blood with 2 cc instead of 1½ cc of distilled water and, therefore, to increase the size of the outer chambers on the slides.

The details of the test considered most satisfactory of those done in this study follow:

FINGER BLOOD PRECIPITATION TEST FOR SYPHILIS

1 Pipette 0.04 cc of defibrinated finger blood into each inner chamber on the 3 by 2 inch glass slide

2 Allow one drop (about 0.015 cc) of 4 per cent sodium chloride solution to fall from a capillary pipette into each inner chamber containing blood and rotate the slide quite vigorously for one minute

3 Allow two small drops (each about 0.007 cc) of sensitive antigen emulsion to fall from a capillary pipette into the first blood salt solution mixture. In the same manner allow a similar quantity of very sensitive antigen emulsion

TABLE IV

CLINICAL COMPARISON OF FINGER BLOOD TEST BY TECHNIC DESCRIBED IN THIS PAPER AND SERUM TESTS FOR SYPHILIS WITH THE SAME ANTIGEN

	FINGER BLOOD TEST (TECHNIC DESCRIBED IN THIS PAPER)						HEATED SERUM SLIDE PRECIPITATION TEST						HEATED SERUM WASSERMANN TEST					
	VERY SENSITIVE EMULSION			SENSITIVE EMULSION			VERY SENSITIVE EMULSION			SENSITIVE EMULSION			VERY SENSITIVE EMULSION			SENSITIVE EMULSION		
	AGREE MENT	DISAGREE MENT		AGREE MENT	DISAGREE MENT		AGREE MENT	DISAGREE MENT		AGREE MENT	DISAGREE MENT		AGREE MENT	DISAGREE MENT		AGREE MENT	DISAGREE MENT	
Syphilitic sera this series ± to +++++ in one or more tests	75 92 59%	6 7 41%		72 88 89%	9 11 11%		75 92 59%	6 7 41%		76 93 83%	5 6 17%		61 77 22%	18 22 78%		44 56 41%	34 43 59%	
Syphilitic sera this series negative to +++++	85 93 41%	6 6 59%		82 90 11%	9 9 89%		85 93 41%	6 6 59%		86 94 51%	5 5 49%		71 79 78%	18 20 22%		51 61 36%	34 38 64%	
Syphilitic and nonsyphilitic sera this series (over 65% positive syphilitic sera)	115* 95 04%	61 4 96%		112 92 56%	9 7 44%		115 95 04%	6 4 96%		116 95 87%	5 4 13%		101 84 87%	18 15 13%		83 70 94%	34 29 06%	
Approximate result if series contained 20% positive syphilitic sera (high average)	Approx 399	6		Approx 396	9		Approx 399	6		Approx 400	5		Approx 377	18		Approx 356	34	
	98 52%	1 48%		97 78%	2 22%		98 52%	1 48%		98 77%	1 23%		95 44%	1 56%		91 28%	8 72%	

*115 to 6 and 2 in clinically doubtful cases = 123 total tests

Evaluation

Negative in all tests with syphilitic sera or nonsyphilitic sera = Agreement

± to ++++ in all tests with syphilitic sera = Agreement

Negative in one test ± to ++++ in one or both other tests with syphilitic sera = Disagreement.

to fall into the second blood salt solution mixture. Rotate the slide with moderate vigor for four minutes.

4. Allow 2 cc of distilled water to fall from a 25 cc pipette (graduated in tenths) into each mixture (the mixtures spill over to the outer rings) and rotate the slide very gently for one minute to completely dilute the blood.

5. Read the results at once or at any time for fifteen minutes after laking is complete, through the microscope at a magnification of about 100 times (low power 16 mm objective, eyepiece 10X or 12½X) and record in terms of pluses according to the degree of clumping and the size of the clumps.

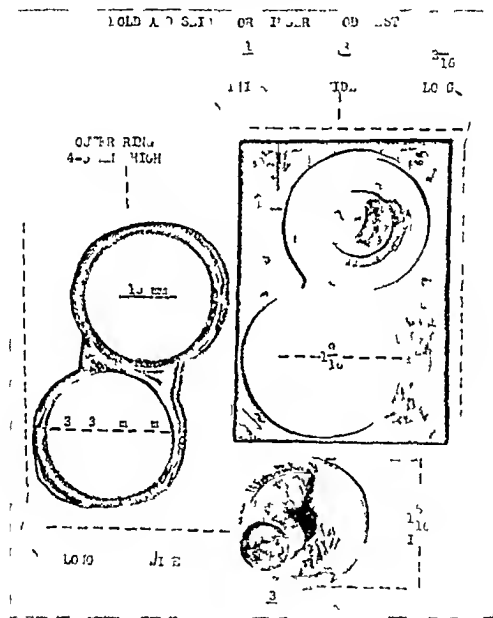


Fig 1

Six sensitive and six very sensitive tests may be done at one time with proper holders.

MATERIALS FOR THE FINGER BLOOD PRECIPITATION TEST FOR SYPHILIS

These are described in detail and illustrated in the first report.¹ Further experience with the test has led to the use of more distilled water in laking the blood. Accordingly, slides with larger wax outer rings than previously described are used. (See illustration of mold and slide.)

The pipette for the 4 per cent salt solution is a capillary pipette made from

glass tubing 8 to 10 mm in diameter with capillary tube about $\frac{3}{4}$ mm in diameter delivering a drop (by gravity) about 0.015 cc (33 drops per $\frac{1}{2}$ cc)

The pipette for the antigen emulsions are similar pipettes with tubes about $\frac{5}{8}$ mm in diameter delivering drops (by gravity) about 0.007 cc (71 drops per $\frac{1}{2}$ cc)

Four per cent salt solution This is made with sodium chloride (c p or Reagent Merck) and distilled water of P_H 5.4 to 6.0. The P_H of the distilled water is determined simply by adding to 0.25 cc of it a drop of chlorophenol red indicator (La Motte). Water satisfactory for use gives a light to medium reddish-purple color. Water of P_H 5.2 or less gives a yellow color with the indicator and is not as satisfactory.

Antigen The antigen is a lipid obtained by precipitation in acetone at 50° C to 37° C of concentrated absolute alcohol extract of beef heart muscle powder (Difco). The details of its preparation are given in a previous report.²

Antigen emulsions containing more cholesterol than formerly employed have been found more satisfactory for the finger blood test. Likewise it has been found more convenient to increase the sensitivity of the antigen emulsion by the addition of a small quantity of acetic acid than to heat the emulsion at 50° C for twenty minutes as previously. The antigen emulsions considered best for the finger blood test are as follows:

<i>Sensitive Antigen Emulsion</i>		<i>Very Sensitive Antigen Emulsion</i>	
0.85 cc	Distilled water (P_H 5.4 to 6.0)	2.0 cc	of sensitive finger blood test antigen emulsion
1.25 cc	of 1 per cent cholesterol (Pfanzahl C P) in absolute ethyl alcohol (99+ per cent) (Prepared in about forty-five minutes by placing in an oven at 50° to 56° C and shaking gently a few minutes at fifteen minute intervals)	0.2 cc	$\frac{1}{2}$ per cent glacial acetic acid (Distilled water P_H 5.4 to 6.0)
0.1 cc	Antigen		
2.2 cc	of 0.85 per cent sodium chloride (c p or Reagent, Merck) solution (made with distilled water P_H 5.4 to 6.0)		

The sensitive antigen emulsion for the finger blood test has the same formula as the very sensitive antigen emulsion for the microscopic slide precipitation test with serum and like it is prepared as follows:

Into a one ounce bottle, 0.85 cc of distilled water is pipetted.

The bottle is held at an angle and the 1 per cent cholesterol in absolute ethyl alcohol (99+ per cent) is allowed to run along the side of the neck of the bottle.

The bottle is gently rotated from the neck for twenty seconds.

The bottle is held at an angle again and 0.1 cc of antigen is pipetted against the side of the neck from a 0.2 cc pipette (graduated in thousandths).

The bottle is promptly stoppered with a cork and shaken fairly vigorously (the fluid thrown from bottom to cork and back) for one minute.

Lastly, the 0.85 per cent sodium chloride solution is allowed to run in quite rapidly, the bottle is stoppered again and shaken as previously for one minute.

The emulsions, when examined through the microscope, at a magnification of about 100 times, show numerous very fine particles but no clumps whatever.

One half hour after the preparation of the sensitive finger blood test emulsion, 2 cc of it is pipetted into a one ounce bottle

Two tenths cc of 14 per cent glacial acetic acid is then pipetted against the side of the neck of the bottle. The bottle is stoppered well with a cork and shaken quite vigorously as before for one minute. The acidified emulsion is the very sensitive finger blood test emulsion.

The emulsions are thoroughly satisfactory for use for six hours after their preparation. After this time there is a slight steady decline in their antigenic power.

The defibrinated finger blood keeps well in the humidor in the refrigerator for at least twenty four hours. Sixty eight specimens kept in the refrigerator in sealed glass tubes made from 3 mm glass tubing (about 2 mm inside diameter) gave results as late as six weeks later differing but little from those with the blood when first obtained.

CONCLUSIONS

1 The simple precipitation test for syphilis with small quantities of defibrinated finger blood described previously has been found in 1000 tests to be more sensitive than the serum Wassermann test with the same antigen and almost as sensitive as the serum microscopie slide precipitation test. No false positive reactions occurred in any of the tests in this series. In 121 cases, the finger blood test performed with the slight changes noted above, gave results agreeing with the clinical condition of the patients as often as those of the serum microscopie slide precipitation test and more frequently than those of the serum Wassermann test.

2 The finger blood precipitation test for syphilis is satisfactory for use in the diagnosis of syphilis in all cases, and since it requires but a small quantity of comparatively easily obtainable blood, it is particularly useful in the diagnosis of syphilis in infants and children. Furthermore, after satisfactory blood typing and matching the finger blood test for syphilis since it is reliable and takes but about eight minutes to do, is an excellent method for determining the suitability of blood donors immediately before transfusion.

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MODIFICATIONS IN THE METHOD FOR THE DETERMINATION OF CHOLESTEROL IN BLOOD*

BY S L LEIBOFF, A M NEW YORK CITY

IN 1924 the author¹ simplified the method for determination of cholesterol in blood by absorbing the blood on filter paper and extracting the cholesterol from the filter paper with chloroform. A special extraction tube was

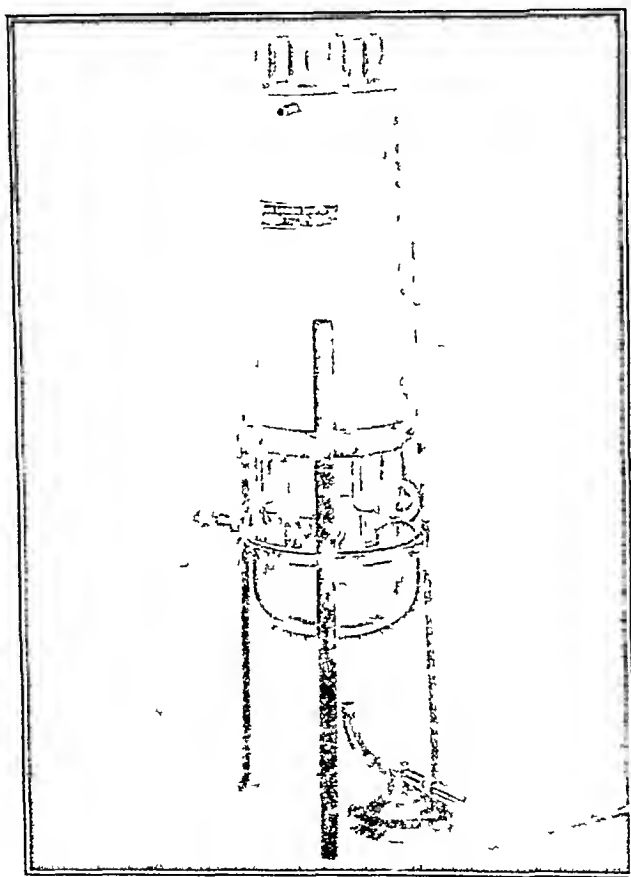


Fig 1

devised² in which the whole operation was performed without transferring material thereby avoiding possible loss. For the extraction a multiple condenser³ was used. In this condenser six samples of blood could be extracted simultaneously. The condenser is of very simple construction and occupies a small space in the laboratory.

*From the Biochemical Laboratory of Lebanon Hospital New York
Received for publication December 19 1929

A number of improvements have now been made in the apparatus which make the extraction even more accurate and allow the easier handling of the apparatus.

The most important change was made in the condensing tube C (Fig 2). Originally this tube was constricted at the bottom, the diameter of this constricted portion was much narrower than the rest of the tube. This was found to be faulty in that it did not always allow the free passage of chloroform, thus causing some of the chloroform to collect in the condensing tube above the constricted portion. Since the constricted parts of the different condensing tubes differed somewhat in diameter no uniform extraction per unit of time was possible with different condensing tubes, since the amount of extraction depends upon the number of drops of chloroform passing through the filter paper.

This difficulty was entirely and satisfactorily overcome by eliminating

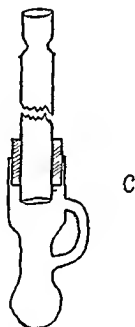


Fig 2

the constricted portion, the whole tube being of the same diameter. A large number of these improved condensing tubes were tested as to the number of drops passed per unit interval of time and very uniform results were obtained. Also a large number of bloods were extracted and cholesterol determinations performed in triplicates; very good checks were obtained. After extraction of the filter papers for half an hour no more cholesterol could be extracted, thus showing that the first extraction was complete.

Another improvement was made in the substitution of a small copper water bath for the large glass beaker. By using a beaker it was necessary to raise the whole apparatus when extraction tubes had to be placed in or removed from the water bath. This is no longer necessary since the copper water bath is held by an iron ring which may be raised or lowered by means of a clamp attached to one of the legs of the tripod, as shown in Fig 1. The low form gas burner was found more suitable than the electric stove, though the electric stove may be used.

In preparing the blood for extraction it is of utmost importance to use the right grade of filter paper *. An extraction paper to be efficient should be very porous and not too hard, under such conditions the blood is exposed to a large number of pores in the paper and is easily absorbed. The large pores also allow the free passage of the chloroform, thus producing efficient extraction. When a filter paper is too hard its porosity is greatly diminished and a greater resistance is offered to the passage of the extracting fluid, as a result some drops of fluid will accumulate on the surface of the paper without penetration through the paper, thus producing inefficient extraction.

DETERMINATION OF CHOLESTEROL

The method for the determination of cholesterol in blood is the same as described in the original paper ¹. Twenty five-hundredths c.c. of well-mixed oxalated blood is placed on a filter paper disc. This is best done by placing the paper disc over the cavity of a porcelain plate such as is used for titration with outside indicators, and distributing the blood from a small pipette over the paper disc by moving the tip of the pipette over the surface of the paper. In this manner the blood is spread over a large area of the disc. The placement of the filter paper over the cavity of the porcelain plate prevents the loss of blood from the under surface of the paper disc were it to penetrate the paper.

The disc containing the blood is now allowed to dry at room temperature for about ten minutes. It may be dried for a short while in the incubator at 37° C, but no higher temperatures should be used as the heat will so affect the paper as to give lower values for cholesterol.

An extraction tube is then filled with about 5 c.c. of pure dry chloroform and the disc containing the blood is picked up with a pair of forceps and placed in the tube containing the chloroform. The same forceps may be used to push the disc down into its place and straighten it out so that it rests over the constricted portion of the tube. One must observe that the disc should lie well beneath the opening of the side arm so as not to obstruct it, as this would prevent the free passage of the chloroform. The tube is then attached firmly to the stopper of the condensing tube and the water-bath is raised to such a height that the extraction tube is immersed in the water to the level of the chloroform. Extraction is continued for half an hour from the time the chloroform begins to boil. When the extract is cool chloroform is added exactly to the 5 c.c. mark on the tube.

In a similar tube are placed 5 c.c. of cholesterol standard (5 c.c. of chloroform containing 0.4 mg. cholesterol).

To each tube is then added 2 c.c. of dry acetic anhydride. The tubes are placed in a beaker of cold water and 0.1 c.c. of concentrated H₂SO₄ added to each tube. The tubes are then removed from the cold water, tightly stoppered with clean dry cork stoppers and inverted to mix the contents. They are then placed in a dark place for ten minutes to allow the color to develop.

*The filter paper discs may be obtained from The Empire Laboratory Supply Company
New York

and are compared in the colorimeter. The colorimetric matching is done in a somewhat darkened room.

Calculation of Results

$$\frac{S}{R} \times 160 = m, \text{ cholesterol per 100 c.c. of blood}$$

S = reading of standard

R = reading of unknown

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COLORIMETRIC DETERMINATION OF SMALL AMOUNTS OF ARSENIC IN BIOLOGIC MATERIAL*

By E. H. MAICHLING, PH.D., AND F. B. FLINN, PH.D. NEW YORK CITY

EARLY in our investigation as to the effect of arsenic on the skin we were faced with the fact that the March method was unreliable when used as a quantitative method in determining the minute quantities of the metal present in the samples of blood, spinal fluid, and scales which were received in the laboratory. Observations showed that these results might be as much as 100 per cent out of the way on some of our samples. This led to a study of the literature of the subject. To those interested in such a survey we recommend the papers by Minot,¹ Kleinmann and Pangritz² who have published a comprehensive study of the methods suggested previous to 1927.

Recently Truog and Meyer³ proposed an improvement on Deniges' colorimetric method for phosphorus and arsenic by means of stannous chloride as a reducing agent. They state that they had been unable to find a systematic study as to the effects of the concentration of sulphuric acid, ammonium molybdate and stannous chloride on the intensity of the color produced. Evidently the work by Kuttner and Cohen⁴ on this problem had been overlooked by them.

Deniges⁵ pointed out the sensitiveness of the reaction between ammonium molybdate and stannous chloride. Huttig⁶ studied this reaction and concluded that it could be used for the detection and estimation of stannous chloride. Deniges⁷ in a later paper discusses the different molybdenum blues in the presence and absence of such reducing reagents as stannous salts, copper, mercury, tin, aluminum, zinc and hydroquinone. He discusses the differences between the stable and unstable molybdenum blues and came to the conclusion that an ammonium molybdate-sulphuric acid solution which had been reduced

*From the Laboratory of Industrial Hygiene, Columbia University.
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by means of copper filings to a yellow solution containing MoO_3 was the best reagent for the detection and determination of arsenic⁸

When trying this method out in our laboratory, we discovered that the amount of copper present in the reduced ammonium molybdate sulphuric acid solution affected the results. It is difficult to control the amount of copper which would be brought into solution because it depends on the surface of the copper filings exposed.

After studying the various reducing reagents, we came to the conclusion that hydrazine sulphate was the best reagent for the purpose. It permits the use of definite quantities of the chemicals employed and produces the yellow solution resembling Deniges' reagent containing the lower oxides of molybdenum. The ammonium molybdate sulphuric acid solution reduced by hydrazine sulphate will give a definite though faint blue color when as little as 0.25 milligram of pentavalent arsenic or phosphorus is present. The blue color is permanent for twenty-four hours or longer which is an advantage.

Our improvement of the Denigès method consists in the use of hydrazine sulphate as a reducing reagent which reduces the ammonium molybdate sulphuric acid solution, decomposing during the reduction into gaseous products which are eliminated, before it is added to the solution containing arsenic or phosphorus. An excess of the reducing reagent is avoided which is necessary as it prevents the reduction of the pentavalent arsenic. (See Kubina⁹) The method depends on the reaction between the pentavalent arsenic and the lower molybdenum oxides. Wu¹⁰ discusses in a very able manner the reactions which take place between molybdenum and pentavalent phosphorus or arsenic.

METHOD

Our method of analysis is briefly as follows:

The dried and weighed sample is destroyed in a Kjeldahl flask by means of concentrated sulphuric and nitric acids in the presence of a drop of concentrated copper sulphate. Care must be taken to avoid charring for as shown by Kesten¹¹ this will result in a loss of arsenic. With some material it has been found advantageous to use a little peroxide of hydrogen to assist in the combustion. After the wet combustion has been completed, the arsenic is distilled off using the same flask as was used in combustion for the distillation flask. The procedure is that described by Bang.¹² The distilled arsenic chloride is caught in a receiving cylinder containing an oxidizing mixture made up of 15 c.c. of concentrated nitric acid and 5 c.c. of bromine water to convert the arsenic to the pentavalent form. We have found it advantageous to use the Allihn form of condenser and place between the condenser and the cylinder a connecting safety bulb into which some of the oxidizing solution has been drawn. The distillate is placed in a porcelain casserole and evaporated to dryness on a water-bath.

Care is taken not to carry the distillation to the point where SO_2 is carried over. If this should occur, the solution must be neutralized with NaOH after evaporation then made slightly acid with sulphuric acid using phenolphthalein as an indicator. It also requires a new standard arsenic solution, prepared in exactly the same way.

The solutions required for the colorimetric determination are prepared as follows

A A 1 per cent hydrazine sulphate solution

B Ammonium molybdate sulphuric acid solution This is prepared by mixing 1 volume of 10 per cent ammonium molybdate solution with an equal volume of concentrated sulphuric acid in 7 volumes of water To obtain a perfectly colorless solution the ammonium molybdate solution must be added to a cold solution of the sulphuric acid in water

C To prepare the molybdate solution 10 cc of Solution B and 2 cc of Solution A are placed in a test tube and immersed in boiling water for exactly one and a half minutes The solution should now be yellow and while still warm is poured back and forth between two test tubes to assist in the removal of the gas bubbles which are formed during the reduction The solution free from gas bubbles is cooled and placed in a glass stoppered container for use This solution keeps for a week Before being used it should be checked by adding 0.6 cc of the solution to 9 cc of water and heating on a hot water bath for five minutes No color should develop On addition of 5 cc of a standard solution of arsenic a light blue color appears

D Standard arsenic solution A standard arsenic solution is prepared from chemically pure pentavalent arsenic in such a way that each cc of the solution will contain 0.001 of a milligram of arsenic This solution should be kept in a glass stoppered bottle in a cool place

Details of colorimetric determination Nine cc of water and 0.6 cc of reduced molybdate reagent are added to the usually colorless dry residue from the distillate in a casserole and the casserole is covered with a watch glass and placed on an actively boiling water bath simultaneously with another casserole containing 5 cc of the standard arsenic solution to which 4 cc of water and 0.6 cc of the reduced reagent C have been added The standard and unknown samples are kept in boiling water for five minutes They are then allowed to stand at room temperature for at least half an hour and then transferred to a 10 cc graduated test tube and when cold distilled water is added to bring the solution up to the 10 cc mark

The solutions are compared in a micro colorimeter The amount of reagent used gives accurate results over a range from 0.25 micrograms up to 20 micrograms To determine larger quantities of arsenic, one must add more of the reagent and a correspondingly larger amount of water When larger amounts are expected in specimens of biologic material, then the distillate should be made up to a definite volume and only an aliquot part of it used for the distillation and subsequent colorimetric determination

It is necessary to point out the importance of repeated blank tests for all the reagents used throughout the complete procedure for it has been our experience that even the so called arsenic free chemicals frequently contain small amounts of arsenic Erroneous results would be obtained without this correction when one is determining the small amounts generally present in such biologic specimens as blood and spinal fluid (Brahme¹³) The reagents used in our work gave values for the blank up to 3.84 micrograms for the amounts used for a complete analysis

SUMMARY

- 1 Hydrazine sulphate is introduced as the reducing reagent
- 2 The reduction of the ammonium molybdate sulphuric acid solution is carried out previous to the addition to the solution containing the arsenic or phosphorus
- 3 The reducing agent, hydrazine sulphate, is completely decomposed during the reaction and the gases expelled leaving only the lower oxides of molybdenum which combines with the pentavalent arsenic, producing a blue color which is directly proportional to the amount of arsenic present

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A SMALL PORTABLE ARTIFICIAL PNEUMOTHORAX APPARATUS*

BY MINAS JOANNIDES M S M D CHICAGO ILL

COMPRESSION therapy in certain cases of pulmonary tuberculosis is now beyond the point of experimental stage. In sanatorium and hospital patients artificial pneumothorax can be induced very easily regardless of

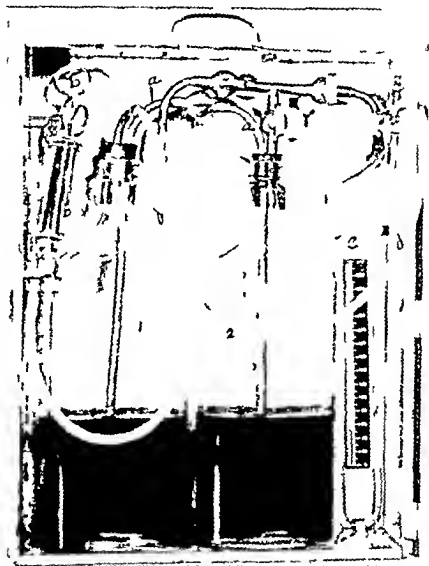


Fig. 1

what apparatus one may use for this purpose. If however one treats ambulatory patients it becomes necessary to use a handy portable outfit that would not occupy much space in the office or in the automobile. The apparatus described here was designed with this purpose in mind.

It is made up of a very lightweight wooden box which is 14 inches long, 11 inches wide, and 3 inches deep. It contains two 1000 cc bottles that are connected to each other by means of a brass tubing "a" (see Fig. 1). By

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SUMMARY

1 Hydrazine sulphate is introduced as the reducing reagent

2 The reduction of the ammonium molybdate sulphuric acid solution is carried out previous to the addition to the solution containing the arsenic or phosphorus

3 The reducing agent, hydrazine sulphate, is completely decomposed during the reaction and the gases expelled leaving only the lower oxides of molybdenum which combines with the pentavalent arsenic, producing a blue color which is directly proportional to the amount of arsenic present

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

A SIMPLE OBJECT FINDER Bowen E. C. Science 70 1600, 1900

The method consists simply of the use of a blank slide on one side of which is pasted a fairly thin sheet of gummed paper trimmed accurately along the edges of the slide. On either end of this a circle is drawn with a five-cent piece (2.2 cm in diameter). When a microorganism or another object of particular importance which is to be photographed or demonstrated later is found a rough sketch of the high power (H.P.) of oil immersion field is drawn in the circle at the left end and a rough sketch of the low power (L.P.) field in the circle at the right end of the paper covered slide. In each case the object of interest is drawn and its position relative to particularly conspicuous material is indicated. Examples of such conspicuous material are masses of pigment, partitions, margins of action, ganglion cells, round cells (P.C.), blood vessels (B.V.) and central canal (C.C.). The same is done if the slide preparation is a smear instead of a section.

The slide in which a field of special interest has been found is then removed from the mechanical stage and the paper covered slide on which the high power and low power sketches have been drawn is put in its place. It is necessary to make sure that the mechanical stage is not jarred and that the slide is in the proper position. A dot is then made with pen and ink as nearly as possible in the center of the bright area transmitted from the condenser. The low power lens is then focused on the dot and its situation in the field noted. The paper covered slide is then removed and the situation of the dot in relation to the field is indicated with a small circle around the dot. The paper covered indicator slide is then numbered to correspond to the slide containing the prized field and both are filed away together for future reference. If more than one interesting field is found in the same slide a number of additional indicator slides may be used.

To find the particular field or fields later the indicator slide is put in place and the dot brought to the relative position in the low power field as indicated by the circle surrounding it. The slide containing the object is then put in its place, the low power field is oriented and the object of interest brought to the center of the field. The high power or oil immersion lens is then turned into position and the prized object is almost always within the field, and if not it can readily be located.

GNORREHEA Recent Advances in the Treatment of White C. and Winter H. G. J. Proc. Army M. Corps 53 200 1929

Exhaustive and extensive considerations of the biochemistry and metabolism of the gonococcus showed that when the gonococcus or any other organism was grown on a medium rich in nucleo-protein it tended to produce polar bodies which were loosely attached to the organism, the bodies turn black with Neisser's stain and are easily distinguishable. They were found to consist of equal parts of alpha-nucleo-protein and beta-nucleo-histone, the latter portion being soluble in 2 per cent salt solution. The culture media used were found to be of exceptional value and are described below. The routine preparation of the vaccine is as follows. Large 6 x 1/4 inch tubes of the nucleo-protein medium are inoculated with gonococcal culture and are incubated for twenty-four hours. In order to insure uniform growth, the same sized tubes sloped to the same angle and a standard inoculating loop are used. After twenty-four hours each tube is washed with 1.5 c.c. of 2 per cent saline carbolized with 0.5 per cent phenol, a separate pipette is used for the addition of the saline and for removal of the emulsion from the tubes thus insuring that the stock carbolized saline is not con-

terminated with gonococcal bodies which might autolyse on standing and release their endotoxin. The emulsion is put in vaccine bottles in quantities of 25 cc. Periodical counts show the average content to be 7,000 $\times 10^6$ per cc. These bottles are put, with the least possible delay, in a high speed centrifuge giving 9,000 revolutions per minute and are "swung" for about four minutes. After centrifugation it will be noted that the contents have separated into three layers, a lower grayish layer consisting of the bodies of the gonococci, a middle cream colored layer of the alpha nucleo protein element of the polar bodies and a clear supernatant fluid which is a saturated solution of the beta nucleohistone in a 2 per cent carbolyzed saline. This clear fluid is pipetted off and put in vaccine bottles ready for use.

The vaccines are given intradermally.

The greatest care must be exercised to carry out the whole process of production under the strictest aseptic precautions as sterilization by heat will only result in the destruction of several important properties.

APPARATUS

1 Test tubes. The most convenient size is 6 inches by $\frac{3}{4}$ inch. These are boiled in the following solution:

Potassium chromate	50 gm
Sulphuric acid, commercial	60 cc
Tap water	500 cc

for two hours and then brushed and rinsed in hot water three or four times and left to soak in water for two hours, next dried in a hot air oven, plugged and sterilized at 160° C for one hour.

2 Filler. This is sterilized at 120° C for one hour.

3 Flasks. Fill with strong solution of potassium permanganate and leave for twenty four hours, wash with hot water followed by commercial hydrochloric acid. When all stains are removed the flasks are rinsed in hot water to render them acid free, dried in the hot air oven, plugged and sterilized at 160° C for one hour.

MEDIA

There are two types of media employed (1) Isolation medium (2) Nucleic acid medium.

The isolation medium is essentially a nutrient serum agar, while the nucleic acid medium contains in addition a thymus nucleic acid base. They both contain an alkaline autolysate of ox heart.

A Alkaline Autolysate of Ox Heart—Obtain the hearts fresh, free them from all trace of fat and fibrous tissue, cut the meat into small cubes and mince in a sterile mincer. Weigh the minced heart and place in a sterile pan. To every gram of meat add 1 cc of distilled water and, in the case of fresh hearts, 40 cc of N/1 NaOH to every liter of water added. In the case of frozen hearts 42.5 cc of N/1 NaOH.

Leave the whole in the ice chest overnight to macerate. Next morning strain through gauze in 500 cc bulks into liter flasks. Steam for quarter of an hour to allow coagulation to take place.

Strain again and steam for fifteen minutes on the first day, five minutes on each of three successive days.

B Agar Base—

Witte's peptone	6 per cent
Agar fiber	6 " "
Sodium chloride	1 " "
Sodium phosphate	1 " "
Agar dist	qs

This base is made up in 200 cc bulks in 500 cc flasks and is placed in a steamer until the agar is melted, then to each add 0.3 per cent of glucose and steam for a further fifteen minutes.

The base is made up the day before the batch is to be tubed off.

C Herring Roe Extract—Take ripe sperm from herring grind it into a paste with distilled water, adding to every gram of roe 1 cc of water. Strain through gauze steam for half an hour on three successive days.

In an emergency tinned roes may be used (Noel and Sons are the best), but the resulting yield of bodies is not good.

D Nucleic Acid Base—

Dessicated thymus	4 gm
Nucleic acid	1 gm
Aqua dist	100 cc

Mix in 250 cc flask and steam for fifteen minutes. Adjust the pH to 7.2 as follows.

Add 1 cc of 0.01 per cent phenol red solution and 100 cc of herring roe extract then add N/1 NaOH until the yellow color turns to brownish yellow steam for a further ten minutes. Note. If it is heated too much there is apt to be an alteration in the pH and consequent loss of efficiency of the medium.

I ORDINARY ISOLATION MEDIUM

Steam the agar base (made as in B above but without glucose) until the agar has melted. Steam the heart extract (A above) for five minutes. Mix 200 cc of agar base with 200 cc of heart extract. Tube off 10 cc to a tube and add 0.5 cc of human serum to each tube and slope.

II STANDARD OR NUCLEIC ACID MEDIUM

Take agar base 200 cc and steam until melted. Heart extract 50 cc and steam for five minutes. Nucleic acid base 200 cc and steam for fifteen minutes.

Mix all together tube off 15 cc to a tube and to each tube add 0.5 cc of human serum. Slope.

The following brands of ingredients have been found to give the best results.

Witte's peptone must be Bostock German brand. Sodium phosphite British Drug Houses pure. Nucleic acid Martindale's. Thymus dessicated Willows Francis, Butler and Thompson.

BLOOD STAIN Rees C W Science 71 1831 1930

Rees reports the following method of fixation as enabling the use of Giemsa's stain and iron hematoxylin for the demonstration of intracellular parasites.

After drying the smears are immersed in Schaudinn's alcoholic sublimate solution without acetic acid.

Good results may also be obtained by first placing the smears in a Coplin jar containing about 3 cc of 40 per cent formaldehyde and then treating with the alcoholic sublimate solution. This is especially useful when the work is done where the humidity is high.

SYPHILIS The Controlled Flocculation Test for Michaloff A. Am J Hyg 11 209 1930

The antigen is prepared from fresh real heart freed from fat and blood vessels and passed through a meat grinder. The muscle is put in a large flask and 95 per cent alcohol added in the proportion of 125 cc of alcohol to 100 gm of muscle. The flask is kept at room temperature for twenty hours then the alcohol is filtered off and the muscle spread between several sheets of filter paper and gently pressed to dry it. It is necessary to change the filter paper several times until no more moisture is taken up by it. After about an hour the muscle is sufficiently dry to be put in a large dry flask and acetone added in the proportion of 200 cc of acetone to 100 gm of muscle. A guinea pig is then killed its brain freed from membranes and vessels cut into small pieces and 2 gm of brain are added for each 100 gm. of muscle in the acetone. This is placed in the incubator at 37° C for forty-eight hours and frequently shaken. Filter off the acetone, dry between filter paper as before. Then again pass the muscle brain mixture through a fresh solution of acetone in the same

proportion as before, being sure to use a clean dry flask for the fresh acetone. The mixture should remain in the incubator for forty eight hours and shaken frequently. Again the acetone is filtered off and the muscle brain tissue dried between several sheets of filter paper. The above described steps are merely to remove the anticomplementary substances and are followed by the true extraction. The muscle-brain tissue, dried and hardened by the above mentioned operations is now placed into absolute alcohol in the proportion of 200 cc of absolute alcohol to 102 gm of tissue in a large, dry, well stoppered flask. The flask is kept in the incubator at 37° C for six days, and shaken very often. On the seventh day the supernatant liquid should have a yellowish color. Filter the liquid through two sheets of filter paper and stopper the flask well. The next day filter again. This filtered liquid is the antigen which shows no tendency to self precipitation and in well stoppered dark flasks can be used for more than a year. This antigen is not anticomplementary nor hemolytic in a dilution of 1 to 5 and if properly prepared can be used in a dilution of 1 to 20 with 8.5 per cent saline, or 1 to 30 for the regular Wassermann test without antigen titration. No further manipulation is necessary for its use in the Bordet Gougen reaction.

Technic of the Test—

The antigen is diluted immediately before using in the proportion of 1 cc of antigen to 20 cc of 8.5 per cent saline. It is very important to add the saline to the antigen in drops while shaking constantly.

The hemolysis. A small quantity of complement should be used. The author had best results with 4 hemolytic units. Titration was done with 0.5 cc of a 5 per cent solution of washed sheep cells and 0.5 cc of 1/100, 1/200, 1/400, etc, dilutions of hemolysin in the presence of 0.2 cc of 1 to 10 diluted complement collected twelve hours previously. If 0.5 cc of a 1/3200 dilution of hemolysin produced complete lysis in thirty minutes, we used equal parts of 1/800 dilution of hemolysin and 5 per cent sheep cells as our hemolytic system.

Complement titration. This should be done daily with 1 cc of the fresh hemolytic system and 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 cc of 1 to 10 complement dilution in the presence of 0.5 cc of saline. Ordinarily 0.1 cc or 0.125 cc is the amount necessary to produce complete hemolysis in thirty minutes. This amount of the complement dilution is used in the reaction but of the hemolytic system they use only 0.5 cc (the equivalent of two hemolytic units of complement).

The test. For the flocculation test or the controlled flocculation test take two tubes 1 cm in diameter and 12 cm long. In the first tube put 0.2 cc of the diluted antigen. To both tubes add 0.2 cc of the serum which is to be examined and which has previously been inactivated by keeping it at a temperature of 56° C for thirty minutes. Now add two units of complement to both tubes and sufficient saline to bring the volume to 1.0 cc. The addition of complement is facultative and therefore the test can be done as a flocculation test where complement is not available.

Shake both tubes gently for exactly four minutes. For this purpose a shaking apparatus is desirable in which the racks must be placed in an inclined position. This tends to lessen the forming and bubbling of the solution. Flocculation often occurs, with or without complement. Ability in reading depends upon experience and is facilitated by the use of an agglutinoscope. The reaction becomes more evident after awhile and after twenty four hours at room temperature a large floccule, easily observed, is to be seen in all sera positive to the Wassermann test.

The addition of complement and the control of complement absorption gives a much more definite result, one that is easier to read and more time saving. The latter method requires only about an hour for its completion, and is done as follows. In addition to the directions given above for the preparation of the sample flocculation test with complement one additional step is necessary, i.e., to each of the two tubes is added 0.5 cc of the hemolytic system previously referred to. Shake the two tubes well and let stand in a water bath at 37° C. Hemolysis ordinarily occurs after fifteen minutes. Anticomplementary sera hemolyze slowly or not at all. The result is read five minutes after the control tube shows complete hemolysis as in the ordinary Wassermann test. The end reaction is stable for at least an hour at room temperature. If the reaction is not evident at the end of thirty

minutes or if the control tube does not show marked hemolysis in fifteen minutes the serum should be considered more or less anticomplementary and the results false. The reaction should then be repeated using 4 to 8 complement units. To avoid anticomplementary reactions in sera separate the serum from the clot shaking the serum for five minutes then centrifuge and finally inactivate.

Cerebrospinal fluid is examined by the same technique using 0.5 cc of noninactivated fluid but omitting saline.

AMEBA Study of Stools Cultured for *E. histolytica* Tripoli C J. Am J Med Sc 178 682 1929

The following method was used:

Ingredients of Media (a) Serum Ringer Solution Beef or human blood is obtained. After clotting it is placed for twenty-four hours in a refrigerator. The clear serum is pipetted off and mixed with Ringer's solution in the proportion of one part of serum to eight parts of Ringer's solution. Sterilization is accomplished by passing the fluid through the large type Sutz-Wertz filter which permits the fluid to pass only through the filter pad. Filtration is accomplished by use of full vacuum. The filtered sterile diluted serum is immediately pipetted off with a 50 cc sterile pipette and placed in sterile flasks 50 cc to each flask. The flasks are kept at 5 to 10 C until ready for use. Inactivation of the mixture (at C for thirty minutes) is not necessary.

(b) Starch Rice starch possesses the necessary uniform small grains which are readily ingested by the amebas and further is not easily hydrolyzed when suspended in fluid.

The following method of sterilization was developed which apparently fulfills the requirements and has given entire satisfaction.

The starch is weighed out in 0.5 gm quantities placed in small soft filter paper tubes which are made by rolling a 31 by 5 cm piece of thin filter paper about a pencil and in which the starch is kept in place by lightly crimping the ends of the paper tube. The filled tubes are placed in test tubes which are then plugged with cotton and covered with several layers of wrapping paper which is well tied down over the end of the tube and the whole assembly autoclaved at 15 pounds pressure for fifteen minutes. After autoclaving the paper covering is removed and the tubes are placed in a hot air oven or incubator to evaporate the little moisture that may have condensed upon the walls of the tube.

(c) Acriflavin Solution A 1 per cent solution of acriflavin is prepared and sterilized in the Arnold sterilizer. Acriflavin solution keeps fairly well when protected from light and at a low temperature.

(d) Egg Base Four eggs are emulsified with 50 cc of normal saline and poured into 11 by 15 cm tubes to a depth of approximately 2 cm. The tubes are placed upright in the water bath and heated to 50 C for sufficient time to coagulate to a firm consistency after which they are autoclaved at 15 pounds pressure for fifteen minutes in the upright position. Amebas grow best upon a flat surface.

A. emibly of Media (a) the starch is added (0.2 gm) to the serum Ringer solution (50 cc) by placing the small paper tube into the flask. Slight agitation opens up the paper and liberates the starch.

(b) Acriflavin is added by transferring 0.1 cc of the 1 per cent solution to 50 cc serum Ringer starch which gives an ultimate dilution of 1 to 50,000. Some samples of acriflavin require a greater concentration (1 to 2,000) to retard the growth of unfavorable flora.

(c) Four to 5 cc of sterile serum Ringer's solution plus the starch and acriflavin are then poured into the tubes containing the coagulated egg. The tubed culture media should be stored in the refrigerator until used. At the time of inoculation the media should be warmed to 37 C. An equal number of tubes should be prepared omitting the acriflavin.

The P_H of the prepared media varies from .2 to .8 and needs no adjustment this being the optimum range of hydrogen ion concentration determined for *E. histolytica*.

Dr. Bohlar advocates the addition of 5 gm per liter of potassium acid phosphate to the media which is adjusted to a P_H of .4. The authors have found this to be unnecessary as

the medium is capable of adjusting itself to the original P_H even after forty eight hours of bacterial growth which tend to form organic acids

Method of Inoculating, Examination, and Transplanting Cultures A particle of fecal material about 1 or 2 mm in diameter is picked up on a wooden applicator and is transferred to a tube of plain, and a tube of aerobically charged media which have been warmed to about body temperature

With soft or dysenteric stools, which are more apt to contain vegetative amebas, the material used for inoculation should be obtained fresh from the patient Proctoscopic removals and rectal washings should, similarly, be inoculated immediately

With formed or hard stools, which are more apt to contain cysts, the cold stool will suffice even though it be several days old The same results were obtained with cysts fresh from the patients or with those kept in the refrigerator several days

The tubes are incubated in an upright position at $37^{\circ} C$

Maximum growth is usually seen on the third or fourth day of incubation On the fifth or sixth day the amebas usually die unless they are transplanted into fresh media Under cultural conditions very few amebas encyst, but with frequent transplantation they can be propagated in the vegetative form for an unlimited period

Examination of cultures is made by skimming the debris of starch and bacteria from the surface of the coagulated egg with a capillary pipette having a large lumen (1 mm) which has been scratched and broken to present a square tip Use a Wright's rubber bulb to produce suction A fraction of a drop of material is removed, placed on a slide, cover glass added, and examination made with the 16 mm objective

To transplant the culture a drop of the material is transferred to fresh media that has been previously warmed

BACTERIA Negative Staining for, Dorner, W C Stain Tech 5 25, 1930

Boil 10 gm of nigrosin in 100 cc water about thirty minutes Filter several times through the same filter paper, adding 10 drops of formalin before the last filtration as a preservative Place a small loopful of this solution on a clean slide and add the bacteria with a needle or loop After mixing, spread the mixture a little irregularly on the slide and dry either at room temperature or slowly over a low flame, the preparation being examined in oil immersion without the use of a cover slip

MENINGOCOCCI Preservation of Cultures, Bourguignon, G C Ann Soc Belge de med trop 9 59, 1929

The death of meningococcus in culture, necessitating daily subculture in order to maintain it alive, is attributed to rapid increase of alkalinity and to the presence of too much oxygen An aseptic fluid medium, containing 0.2 per cent glucose, and covered with a layer of vaseline has been used for the preservation of gonococci The glucose by its fermentation retards the increase in alkalinity This medium has been found by the author equally useful for the preservation of both meningococci and gonococci, which have been kept alive for thirty seven days without subculture

INFLUENZA Study of Hemophilus Influenzae, Evans, M J Am J Med Sc 179 177, 1930

The virulence of cultures remains unimpaired for as long as two years if kept at room temperature (18° to $20^{\circ} C$) in infusion broth P_H 7.6 to 7.8 to which is added approximately 7 per cent by volume of sterile defibrinated blood, the whole then heated until it becomes a rich brown color

EOSINOPHILIA In Liver Diet, Mullengracht, E, and Holm, S Am J Med Sc 179 199, 1930

Eosinophilia in liver treatment of pernicious anemia has appeared in a marked and persistent form when the treatment is carried out with raw liver (calf) in large doses As

rule, the eosinophilia has appeared rather suddenly after about four weeks of treatment, and it has reached to high degrees 20, 40 and even 74 per cent. It seems to persist as long as the administration of raw liver is kept up. On treatment with fried liver (oil) or liver extract, the phenomenon has usually been absent and when present in single instances it was in a faint and transitory form.

Control individuals suffering from various other diseases have responded to the treatment in the same manner as have patients with pernicious anemia; as they constantly showed eosinophilia on ingestion of raw liver but not after intake of fried liver or liver extract.

This eosinophilia is to be considered a by-product in the treatment of pernicious anemia with raw liver that has nothing to do with the curative effect of the treatment.

As far as directly observable, the eosinophilia represents a harmless phenomenon.

MORGAN'S BACILLUS Infections Probably Due to D. Aunoy R. Am J Med Sc 178 874 1929

The isolation of Morgan's bacillus in cases of pyrexia, peritonitis and colitis is reported. One strain isolated proved pathogenic for small laboratory animals. Agglutinins were demonstrated for homologous organisms in the blood of all three patients. Observations upon some biologic characteristics of the organism are presented together with a plea in support of Thijssen's position for noninclusion of the organism in the genus *Salmonella*.

DEXTROSE Preparation of Dextrose for Intraperitoneal Injection Shohl A. T. and Beal F. Am J Dis Child 38 943, 1929

Five grams of chemically pure, finely ground dextrose are weighed and placed in a test tube approximately 4 x 3/4 inches (100 mm. by 19 mm.) and the test tube is loosely stoppered with a cotton plug. This is inverted and placed in a wide-mouthed bottle containing from 2 to 50 gm. of granular anhydrous calcium chloride. The bottle is fitted with a cork and autoclaved at 15 pounds (68 Kg.) for twenty minutes. This preparation will keep for at least several months.

For use we make the solution with freshly distilled water. It is taken directly from the still and boiled five minutes (but not redistilled). One hundred cc. is poured on the sterile dextrose which has been placed in the graduated flask to be used for the injection and a 5 per cent solution is thus made. It is given intraperitoneally with the usual precaution at body temperature.

BLOOD CHEMISTRY The Accurate Determination of Chlorine and Iron in Blood and Other Liquids Smirk F. H. Biochem J 22 201 1928

The pipette used is drawn from thick capillary tubing and a length of 2 or 4 cm. is almost sealed off from the main body by means of a constriction. This length should hold from about 0.01 to 0.02 cc. the actual volume from the top to the constriction being determined by weighing mercury from it. For chlorine the pipette is filled with serum, whole blood, corpuscles, urine or sweat and the fluid is delivered to the bottom of a test tube. The pipette is washed three times with distilled water, the washing water being taken from a drop at the end of the burette and the washings added above the liquid in the test tube. The pipette is cleaned, filled with silver nitrate solution of the strength appropriate to the solution under analysis and washed with nitric acid. A little powdered ammonium persulphate is added and the solution is heated until it is clear. It is then cooled and an equal volume of acetone and a drop of saturated iron alum solution are added. The solution is titrated with alcoholic ammonium thiocyanate. The first red flush is taken as the end point. 0.03 cc. thiocyanate added after this should make the solution distinctly red. The acetone diminishes the dissociation of the ferric thiocyanate and makes the end point sharper. For iron in the blood or corpuscles the same pipe is used and concentrated nitric acid and ammonium persulphate are added to the liquid which is then heated, cooled and diluted with 50 per cent acetone. Ammonium thiocyanate solution is added and the color is compared colorimetrically with a standard.

REVIEWS

Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building,
Richmond, Va

*The Common Head Cold**

THIS is an exposition of the causes, prevention and treatment of acute coryza, for popular consumption. It is written from the point of view of the rhinologist.

The author justifies the length of his monograph by the observation that more than ninety million workdays are lost each year on account of this malady, with a total economic cost of four hundred and fifty million dollars. Considerable space is devoted to discussions of anatomy and physiology. The major portion of the volume deals with preventive measures which in the last analysis develops into a discussion of personal hygiene. The author's therapeutic measures for home administration are rational and in keeping with our present day understanding of the problem. He appears to have no pet hobbies.

Botany†

AN INTRODUCTORY textbook on general college botany. Since with many undergraduate students the only formal work in the biologic sciences is the study of botany, these authors have included a general presentation of fundamental biologic principles. In other words this volume is a textbook of biology, in which representatives from the vegetable kingdom are used for illustrative purposes. The illustrations are most abundant and are clearer and more instructive than in many other books dealing with this subject.

Tuberculosis‡

A MANUAL for the use of the patient or his family, on details of home treatment. We can recommend it for this purpose, especially for the patient or family of only moderate intelligence. The more highly educated or intelligent patient will probably want a book of greater detail.

We take exception to the author's statement on page 4, that tuberculosis is not an infectious disease. He explains this statement but the explanation does not satisfy. In the United States there might be some misunderstanding of the statement on page 41, that the bedroom should be preferably on the first floor. Later we find that the higher the bedroom is removed from the ground the better for the patient. This apparent contradiction in the United States, would disappear in England and on the Continent, where our second floor is termed the first floor.

*The Common Head Cold and its Complications. By Walter A. Wells. A.M. M.D. F.A.C.S. Prof. of Otolaryngology, Georgetown University, Washington, D.C. With an Introduction by Hugh S. Cummings. M.D. Surgeon General, United States Public Health Service. Cloth, pages 225. The Macmillan Company, New York, 1929.

†Botany. A textbook for College and University Students. By William J. Robbins, Professor of Botany, University of Missouri, and Harold W. Rickett, Associate Professor of Botany, University of Missouri. Cloth, pages 535. D. Van Nostrand Company, Inc., New York, 1929.

‡Oxford Medical Publications. Tuberculosis. Its Prevention and Home Treatment. A Guide for the Use of Patients. By H. Hynd Thomson, M.D. D.P.H. County Medical Officer of Health, School Medical Officer and County Tuberculosis Officer for Hertfordshire, formerly Medical Superintendent, Liverpool Sanatorium and Medical Superintendent, Consumption Sanatorium of Scotland, Bridge of Weir. Cloth, pages 99. Humphrey Milford, Oxford University Press, American Branch, New York. Third Edition.

NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

*The Clinical Aspects of Venous Pressure**

IN THIS volume Dr Eystor reviews the literature and presents his own experimental work on venous pressure. He brings out the increasing importance of clinical studies of the venous pressure emphasizing that such studies clinically give information concerning beginning congestive failure earlier than any other method. Studies of the venous pressure in cardiacs will therefore probably develop into an important clinical method of control. The author has developed a satisfactory instrument for the clinical determination of venous pressure which will probably come into general use particularly among cardiologists.

*Some Principles of Minor Surgery***

THE author feeling that while major surgical principles have received wide attention in textbooks minor surgery is too often glossed over has devoted a small volume to the latter alone. The work includes the modern surgical treatment of such conditions as clean and infected wounds, abscess infections of the hand, sprains, fractures urinary retention skin tumors hemorrhoids, ingrowing toe nails varicose veins hydrocele and the like.

Pharmaceutical Latin†

A WEST pocket Latin grammar written primarily for the student who has had no previous acquaintance with Latin, in which the vocabulary is almost entirely confined to Latin words used in prescription writing. Chapters are devoted to Latin phrases and abbreviations used in prescriptions to the writing of prescriptions and there is quite a complete Latin English vocabulary which again includes the Latin words customarily employed in prescription writing.

Pharmacology and Therapeutics‡

A POCKET sized handbook on the pharmacology of the common drugs. The field of usefulness of this volume is limited almost entirely to the beginning student of pharmacology. This is indeed the purpose for which its author indicates that it was intended.

Bacteriology, General, Pathologic, Intestinal§

THE third edition of Kendall's work has been widely revised and brought strictly up to date. One may look upon this volume as one on applied bacteriology, rather than pure bacteriology. While it is entirely suited to use as a textbook in the bacteriologic laboratory, the discussions are developed around the diseases caused by bacteria rather than around individual bacteria as a cause for disease. The author devotes considerable space to the chemistry of bacteria bacterial metabolism, pathogenicity, resistance and immunity. There is a very interesting chapter on gastrointestinal bacteriology.

This book should be highly recommended both as a textbook and as a reference manual.

The Clinical Aspects of Venous Pressure By J. A. E. Eystor B.Sc. M.D. Professor of Physiology University of Wisconsin Associate Physician Wisconsin General Hospital Madison Wisconsin. Cloth pages 135. The Macmillan Company New York 1929.

***Oxford Medical Publications. Some Principles of Minor Surgery* By Zachary Cope M.S. M.D. (Lond.) F.R.C.S. (Eng.) Surgeon to St. Mary's Hospital Paddington and to the Brompton Hospital. Cloth pages 159. Humphrey Milford Oxford University Press American Branch New York 1929.

†Aids to Pharmaceutical Latin By G. E. Trease Ph.C. Lecturer in Pharmacognosy University College Nottingham Joint Author of *The Chemistry of Crude Drugs* Cloth pages 168. William Wood and Company New York.

‡An Introduction to Pharmacology and Therapeutics By J. A. Gunn M.D. D.Sc. (Edin.) M.A. (Oxon.) Professor of Pharmacology in the University of Oxford and Fellow Balliol College sometime Examiner in the Universities of Belfast Bristol Cambridge Cardiff Edinburgh Leeds Liverpool London Oxford Sheffield and to the Royal College of Physicians Cloth, pages 220. Humphrey Milford Oxford University Press American Branch New York 1929.

§Bacteriology General Pathological and Intestinal By Arthur Isaac Kendall B.S. Ph.D. Dr. P.H. Professor of Research Bacteriology in the Northwestern University Medical School Chicago Illinois. Third edition thoroughly revised. Illustrated with 103 Engravings and 8 plates. Cloth pages 733. Lea & Febiger Philadelphia 1928.

Organic Chemistry

AS A textbook of organic chemistry this volume covers the usual field and very much in the customary sequence. Its chief interest to physicians lies in its containing details of the chemical constitution and formulas of most of the synthetic organic chemicals that have come into use of late years in the treatment of disease. With new drugs being introduced each year and the older ones being served to us under a variety of trade names, it would seem practically impossible for such a volume to be up to the minute. However practically all of those which have established a definite place for themselves in therapeutics up to the time of printing will be found. It should be of some comfort to the physician to know that somewhere there is a volume to which he can refer to find out precisely what he is injecting into his patient.

Materia Medica†

THIS is essentially a laboratory manual on the analysis of drugs and poisons for undergraduate use and instruction.

Sterilization for Human Betterment‡

THIS is a most interesting report of the results of six thousand sterilization operations in California between 1909 and 1929. All who are interested in this phase of eugenics should have this volume available.

As the authors point out in their introduction, the human race has reached the present stage of its development by the survival of the fittest. The weak and defective have perished. Modern civilization, human sympathy, and charity tend to interfere with nature's plan. The weak and defective are now nursed to maturity and produce their kind. Under nature's law we bred principally from the top. Today we breed both from the top and the bottom, but more rapidly from the bottom.

The authors estimate that 4 per cent of the people of the United States will at some time need the care of an institution for mental diseases. One half per cent of our population has a mentality which never passes that of seven years. One per cent has a mentality of from seven to nine years. The moron class, by far the more dangerous, represents about 5 per cent of the population.

One in twelve of the insane admitted to state hospitals in California since the sterilization law was passed have been sterilized. More recently one in five or six of the new admissions is sterilized. Of 6,255 sterilizations there have been three known failures in the male and four in the female. There were four deaths following operation.

The authors find that sterilization as practiced has no deleterious effects upon the sexual life, indeed actual improvement is reported by one man in seven and by one woman out of three. They find no increase in delinquency. This is of course due in great measure to the careful supervision of paroled patients. Sterilization has not been found to increase the tendency to rape. The authors conclude after careful investigation that sterilization of the type of individual that has been subjected to this treatment incurs no risk of loss of good stock, or of prevention of the birth of genius.

*Organic Chemistry. For Students of Pharmacy and Medicine. By A. H. Clark, Ph. G. E. Sc. M. D. Professor of Chemistry, University of Illinois School of Pharmacy. Member of the General Committee of Revision of the Pharmacopoeia of the United States. Past President of the American Conference of Pharmaceutical Faculties (American Association of Colleges of Pharmacy). Cloth pages 446. D. Van Nostrand Company, Inc. New York 1929.

†Practical Materia Medica. An Introductory Text to the Study of Pharmacology and Therapeutics Designed for Students of Medicine. By Clayton S. Smith, Ph. D. M. D. Professor of Physiological Chemistry and Pharmacology in the College of Medicine of the Ohio State University, Columbus, Ohio, and Helen L. Wikoff, Ph. D. Instructor in Physiological Chemistry and Pharmacology in the College of Medicine of the Ohio State University, Columbus, Ohio. Cloth pages 300. Lea & Febiger, Philadelphia 1929.

‡Publication of the Human Betterment Foundation. Sterilization for Human Betterment. A Summary of Results of 6,000 operations in California 1909-1929. By E. S. Gosney, B. S. LL. B. and Paul Popenoe, D. Sc. Cloth pages 202. The Macmillan Company, New York 1929.

*The Principles of Electrotherapy**

NOWADAYS when one picks up a book on electrotherapy one anticipates finding an elated discussion of the cure of all maladies and, on the flyleaf an advertisement for certain electrical apparatus

The present volume is not that kind. It is the only work that we have come across recently that puts down intelligently, and for what it is worth the modern clinical application of electrotherapeutic measures. We can recommend it to those who are interested in this phase of therapy.

Colloid Chemistry†

THE most interesting presentation of the facts of colloid chemistry known today, readily understandable to anyone who has studied general chemistry. In the first seventy-five pages the nature and general properties of colloids is discussed. The next one hundred and sixty pages describes the practical applications of colloid chemical principles in the various fields of science and the arts covering such diversified subjects as astronomy, perfumes, geology, dyeing, silk, insecticides, sewage disposal, photography, paper, rubber, ice cream, confectionery, glasses, boiler scale, plaster, pharmacology and therapeutics, antiseptics, biology and medicine.

The last portion of the work consists of suggestions for experimental laboratory demonstrations in colloid chemistry using substances which are usually available in every household.

The value of this book lies not only in its relatively nontechnical presentation of the subject but also its availability as a reference manual and its description of practical applications of colloid chemistry.

Practice of Medicine‡

A TEXTBOOK of the practice of medicine by various authors edited by Frederick W. Price, has since its first publication in 1922 gone through three editions with eight printings. The chief change from the first edition reviewed some time ago in these columns consists in the inclusion of new subjects such as tetraethyl lead poisoning, carbon monoxide poisoning, basal metabolism, polyglandular syndrome, internal secretion of the sex glands, intestinal carbohydrate dyspepsia, biliary colic without gallstones, sickle cell anemia, cardiac infarction, the venereal habit, malaria treatment of syphilis, the use of plasmochin in malaria, the laboratory tests for smallpox, diagnostic test of drunkenness, barium therapy and many others of the newer subjects.

This text appears to have successfully supplanted Osler's system in England. Printed on India paper the nearly 1900 pages make a not at all unwieldy volume.

Oxford Medical Publications. *The Principles of Electrotherapy and Their Practical Application*. By W. J. Turrell, M.A., D.M., B.Ch. (Oxon.), D.M.R. & T. (Cantab.), Consulting Physician, Oxford County and City Mental Hospital; Physician in charge of the Physiotherapy Department, Radcliffe Infirmary, Oxford. Major R.A.M.C.T., Late Medical Officer in charge of the Physiotherapy Department, Third Southern General Hospital, Oxford. Ex-President, Electro-Therapeutic Section, Royal Society of Medicine. Honorary Fellow of the American Electro-Therapeutic Association. Honorary Fellow, American Academy of Physiotherapy. Second Edition. Cloth, pages 413. Humphrey Milford, Oxford University Press, American Branch, New York, 1929.

†Industrial Chemical Monographs. *Colloid Chemistry: Principles and Application*. By Jerome Alexander, M.Sc., Consulting Chemist and Chemical Engineer, Past Chairman Committee on the Chemistry of Colloids (National Research Council), Fellow Amer. Assn. for the Adv. of Science and Amer. Inst. of Chemists, Mem. Amer. Inst. of Chem. Engineers, Amer. Inst. Mining and Metallurgical Engineers, etc. Third Edition. Cloth, pages 270. D. Van Nostrand Company.

‡Oxford Medical Publications. *A Textbook of the Practice of Medicine*. By various Authors. Edited by Frederick W. Price, M.D., F.R.S. (Edin.), Consulting Physician to the Royal Northern Hospital; Physician to the National Hospital for Diseases of the Heart, London; formerly Physician and Honorary Pathologist to the Mount Vernon Hospital for Consumption and Diseases of the Chest, and Examiner in Medicine at the University of St. Andrews. Third Edition. Pages 1871. Humphrey Milford, Oxford University Press, American Branch, 1929.

*An Introduction to the Study of Human Anatomy**

THIS is a laboratory guide for dissection, in use at Washington University St. Louis. As we review this volume we are struck with the observation that the student is taught to critically survey and analyze those things which he is inspecting and handling and to do this with an inquisitive point of view.

In other words this volume appears to be an attempt in so far as such is possible in cold type to reproduce the finer type of the human demonstrator in anatomy, who, by pointing out curious and interesting things that might otherwise escape notice is always raising the question of why and wherefore and sending the student to his textbook and reference manuals in a search for the answer. No book can replace such a man, but it should make an excellent supplement to his enthusiastic and inspiring preceptorship.

The Robert Jones Birthday Volume†

A COLLECTION of essays on orthopedic surgery dedicated to Sir Robert Jones on the occasion of his seventieth birthday. Naturally its chief interest will be to orthopedists but there is an especially interesting historical chapter which should have general interest.

Determinative Bacteriology‡

THE first edition of this work was published in August, 1923, and represented an initial effort on the part of the committee on Determinative Bacteriology of the Society of American Bacteriologists to place in the hands of students a detailed key for the identification of species based upon a classification of bacteria into families, tribes, and genera. A second edition of the work appeared in December, 1925, which embodied corrections of the previous work and amended descriptions of individual species. The work has become the standard authority in its field and of great practical value to all laboratories, students, and teachers engaged in bacteriological work.

The third edition of *Determinative Bacteriology* was published in January, 1930. This new volume represents a notable tome, greatly amplified with descriptions for some two hundred additional organisms not included in previous editions. These new organisms fall chiefly in genera *Phytomonas*, *Flavobacterium*, *Pseudomonas*, *Lactobacillus*, and *Bacillus*. Two additional tribes and four additional genera are also recognized. Of great interest is the division of Genus *Eberthella* placing the dysentery and the typhoid organisms in separate groups. Furthermore the new Manual has attempted to arrange the genera of tribe *Bacteriaceae* in a more logical manner and a new key for this tribe has been constructed.

In seven years, through three different editions, the Manual of *Determinative Bacteriology* has gained a place of preeminence as an authoritative work on bacteriologic classification and Professor Bergey and members of the committee are to be congratulated for this very important and most practical contribution to the science of bacteriology. No laboratory or scientific library is complete without it.

*An Introduction to the Study of Human Anatomy. By Robert James Terry, A.B., M.D., Professor of Anatomy in Washington University. Cloth, pages 346. The Macmillan Company, New York, 1929.

†Oxford Medical Publications. The Robert Jones Birthday Volume. A collection of Surgical Essays. Cloth, pages 434. Humphrey Milford, Oxford University Press, American Branch, New York, 1925.

‡Bergey's Manual of Determinative Bacteriology. By David H. Bergey, Professor of Bacteriology, University of Pennsylvania. With an Index by Robert S. Breed. Ed. 3, cloth, 299 pages. Baltimore: The Williams and Wilkins Co.

The Journal of Laboratory and Clinical Medicine

VOL. XV

ST LOUIS, MO., MAY, 1930

No 8

Editor WARREN T VAUGHAN M.D.
 Richmond, Va

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EDITORIALS

Poisoning From Snake Bite in the United States

FROM the earliest time, snakes have been of great interest to mankind. Perhaps in most cases this interest takes the form of an inborn revulsion, but to many their great interest lies in their biologic relation to man and the lower animals. Snakes and lizards represent one great order of living reptiles, of which the other three are turtles, crocodiles and a single species of the ancient group known as Rhynchocephalia. The other orders of this interesting group of vertebrates are all extinct, but they contained some of the largest animals ever on the face of the earth such as the stegosaurs, dinosaurs and ichthyosaurs.

The snakes have furnished zoologists with some of the most fascinating material on which many valuable investigations have been made, yielding answers to many of the interesting and significant problems in embryology, comparative anatomy and phylogeny. The pharmacologist and physiologist both have found valuable material among the snakes, and study of their ven-

omis has made a brilliant chapter in their annals. But apparently not all the problems in herpetology have been solved, and the public health physician in certain localities, and physicians in general, are still confronted with many problems yet to be solved in the biologic relation of snakes to mankind. In these problems many laboratory men have been prominent, and so, recent work in the field should be of interest to pathologists.

The brilliant work of do Amaral in Brazil is now history, and the great benefit of the researches carried out in Sao Palo are well known. In 1926, under his direction, the Antivenom Institute of America was founded with a view to studying the conditions in the United States of America with regard to venomous snakes, and to prepare antitoxic serums against the bites of these snakes.

Fortunately there are but few species of poisonous snakes in the United States. All, with the exception of the coral snake, which has a limited distribution, belong to the same general group of pit vipers, and it was early found that a polyvalent antitoxic serum could be prepared that would be effective against all poisonous North American snakes except the coral snake, which rarely bites a human being. Our poisonous snakes belong to the Crotalidae and consist of the rattlesnake, the cotton-mouth moccasin and the copperhead. Venom extracted from these snakes can be used to prepare an antitoxic serum in horses which is now generally dispensed. The polyvalent nature of the serum is of great practical value. This can be appreciated when one remembers that in the region of Panama and in South America, specific serums have to be administered according to the species of snake involved. It is interesting to note that the anticrotalin serum prepared from North American snakes does not neutralize the venom from Central American rattlesnakes such as *Crotalus terrificus*.

For the first time in the history of this country we have reliable data concerning the incidence of poisoning from snake bite, the effect of treatment, and the results during a specific period of time. Thus, the report of Dr. R. H. Hutchinson of the Antivenom Institute of America for the year 1928 is of tremendous importance. Previous to this report, knowledge of such items was most unsatisfactory. Willson first estimated the number of cases in 1908, from his records he was able to collect 740 cases which dated back as far as 1843 and it is interesting to note that he concluded that poisonous snakes, in particular rattlesnakes, were surely being exterminated and that their bites were becoming more and more infrequent. In this connection one might note that during the War of the Rebellion not a single case of snake bite was reported in the Medical and Surgical History of the War. Some time after 1908 Ditmars attempted to collect data on the cases of snake bite in the United States. From his data he estimated that at least 1000 cases of poisoning from snake bite occur in the United States each year, of which he believed 150 were fatal.

A total of 607 reports of cases of poisoning from snake bite which occurred in 1928 in continental United States, have been collected. Of these it is possible to analyze carefully 458. It further seems likely that these more carefully analyzed cases are the result of poisoning by ten species of snakes,

of which the copperhead leads, with 171 cases, the Texas rattler, with 100 cases, is next. The swamp rattler is given credit for only two cases. A comparatively small number of cases are reported to have been caused by the eastern diamond back rattler, but a large number of bites were caused by the pigmy or ground rattler.

In studying the geographic distribution of poisoning by snake bite, one finds that it follows fairly closely the distribution of the snakes, however, certain discrepancies are to be noted. The distribution of reports of cases is influenced to a large measure by activity in the distribution of antivenom. Thus, the regions around certain cities, such as Houston and San Antonio, Texas, and Atlanta and Savannah, Georgia, become significant. In general, reports are few and scattered from the north Atlantic and north central states, with the exception of Pennsylvania where most of the cases are due to bites by the copperhead. In the southeastern and Gulf states the distribution of cases shows that in Virginia and West Virginia the chief offender is again the copperhead. In North Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana and Arkansas, there is a somewhat different picture because of the inclusion of the cotton mouth moccasin pigmy rattler and a few of the eastern diamond back rattlers. In the Western and Southwestern states, the western diamond back and the prairie rattlers are the predominating forms, with the former predominating in Texas and the latter predominating in Colorado. Even as far north as Montana, bites from the prairie rattler are recorded. In the Pacific Coast and Rocky Mountain states, the Pacific rattler is naturally the predominating form.

As would be expected, the seasonal distribution of cases follows the life history of the snakes. Few cases are reported before the end of April which is correlated with the coming out from hibernation and the activity of the snakes during their period of feeding and breeding which continues until December in the southern states. In the more northern latitudes, the activity of the snakes is limited to the summer season, cases do not appear as early in the year or continue as late. There is some difference in habits of the various species of snakes and with respect to time most reports are rather closely in accord with these specific habits in activity.

The distribution by sex of persons bitten reveals the anticipated result of about twice as many males as females. The distribution by age shows that 50 per cent of the bites occurred in persons under twenty years of age and that in children from two to five years of age, boys and girls were about equally represented.

The situation of the bite, in 57.8 per cent of cases was on the lower extremities, and in 41 per cent of cases on the upper extremities. The circumstances under which the person was bitten determined to some extent the situation of the bite. It appears that 103 persons were bitten while picking up or lifting some object, thirty seven, while reaching under some object, one hundred twenty, when they stepped on or too close to, or fell near the snake, thirty three while fishing or hunting, twenty two while handling captive snakes, and the remainder were bitten under various circumstances.

Invitation to a Symposium on the Kidney in Health and Disease

To Be Held at the University of Minnesota Medical School

Minneapolis, Minnesota, July 7-18, 1930

Preliminary Program

In issuing this invitation to a symposium on the physiology and pathology of the kidney a few explanatory remarks may be appropriate. Attempts to facilitate the correlation of a great number of facts belonging to scattered compartments of human knowledge but all having a bearing on one subject, have become so numerous that this tendency might well be considered one of the characteristics of our times. The high degree of specialization that is associated with intensive scientific development makes such correlations particularly necessary—though they have always been needed and must, in fact, be of the same age as science itself.

We believe that in the field of internal medicine where this symposium belongs this kind of an attempt is not carried out often enough. Whether it ought to become a common occurrence will depend to no slight degree upon the benefit derived by the participants, passive as well as active. It may be pointed out as a characteristic of this symposium that it deals with a relatively small, well defined subject. No attempt will be made to give a presentation of the complete accumulated knowledge of the kidney in health and disease. But, we will try to bring up for discussion those chapters of the anatomy, physiology, and pathology of the kidney where our knowledge has recently been extended in an important way together with other chapters where progress has been difficult to achieve, but where investigative efforts are intense.

Opening Address Dr A J Carlson, University of Chicago

Form and Function of Renal Tubule Dr G Carl Huber, University of Michigan

The Comparative Anatomy of the Kidney Dr E K Marshall, Johns Hopkins Medical School, Baltimore, Md

An Analysis of the Growth of the Human Kidney Dr R E Scammon, University of Minnesota

Further Anatomic Considerations Dr C M Jackson, University of Minnesota

After the foundation laid on the first day, the program will run along three parallel lines as follows:

A Clinical Consideration of Kidney Diseases

Clinics on kidney disease, beginning Tuesday, July 8, at 8 00 A M

Lectures at various other times

B Physiology of the Kidney

Lectures, beginning Tuesday, July 8, at 9 30 A M

C Pathology of the Kidney

Lectures, beginning Tuesday, July 8, at 2 00 P M

D Round table discussions every second day for the correlation of the material presented during the preceding days

Clinic Prof F Volhard, Frankfurt on the Main

The Comparative Physiology of the Kidney Dr E K Marshall

The Physiology of the Glomerulus Dr A N Richards, University of Pennsylvania

General Pathologic Consideration—Glomerulonephritis Dr E T Bell, University of Minnesota

The Physiology of the Tubules Dr H L White, Washington University, St Louis, Mo

The Physiology of the Tubules Dr R N Bieter

- Theory of Kidney Function—The Tubules—Threshold Substances Dr Poul B Rehberg The Zoophysiological Laboratory University of Copenhagen
- Chronicity and Progression in Nephritis Dr Winfield T Longcope Johns Hopkins Hospital
- The Use of Urea and Creatinine in Testing the Function of Diseased Kidneys Dr Poul B Rehberg
- The Phenolsulphonephthalein Test Dr L G Rowntree The Mayo Clinic Rochester, Minn
- Albuminuria and Plasma Proteins Dr H Berglund University of Minnesota
- The Colloids of Blood Plasma the Hofmeister Series and the Hydrogen Ion Concentration Dr A D Hirschfelder University of Minnesota
- Edema Dr L Leiter, University of Chicago
- Edema Dr Poul B Rehberg
- Hypertension Statistical Considerations Dr Harold Diehl University of Minnesota
- Hypertension Dr McN Wetherby University of Minnesota
- Hypertension Dr E T Bell
- The Retinal Changes in Bright's Disease and in Hypertension Dr H P Wagoner, The Mayo Clinic Rochester Minn
- Uremia Prof F Volhard
- Clinic on Hypertension Dr C E Fahr University of Minnesota
- The Retinal Changes in Bright's Disease and in Hypertension (continued) Dr H P Wagoner
- Clinic on Hypertension Prof F Volhard
- Fundamental Studies on Diuretics Dr R N Bieter
- The Effects of the Nerves on Glomerular Bloodflow Dr R N Bieter
- The Effect of the Nerves on Kidney Function Dr L G Rowntree
- Surgery of the Kidney in Bright's Disease Prof F Volhard
- Clinic on Polycystic Kidney Prof F Volhard
- The Clinical Uses of Diuretics Dr N M Kneth The Mayo Clinic Rochester Minn

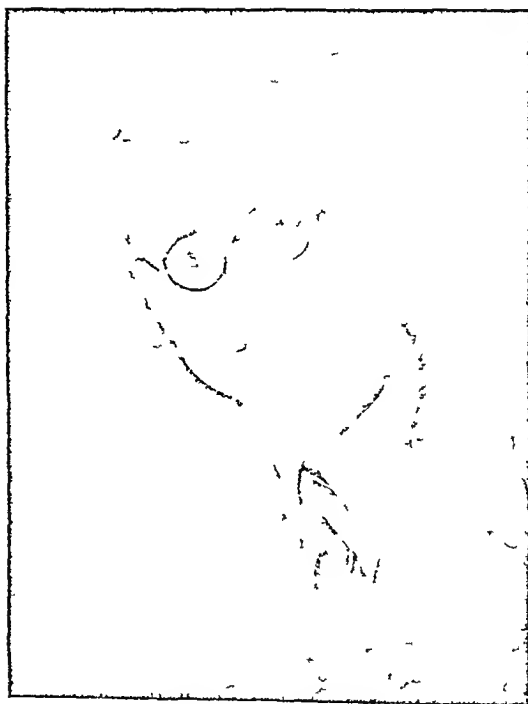
The University asks all those who attend this symposium to register There will be no registration fee

The University will try to provide dormitory accommodations at a reasonable price during the symposium if registration is made before June 1

Hotel accommodations will be arranged for if registration is made before the opening of the symposium

All correspondence in regard to the symposium may be addressed to the Symposium University Hospital, Minneapolis Minnesota, or direct to Dr Hilding Berglund University Hospital Minneapolis, Minnesota

The final program, including a brief synopsis of each lecture will be issued before June 1



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Dallas, Texas
President

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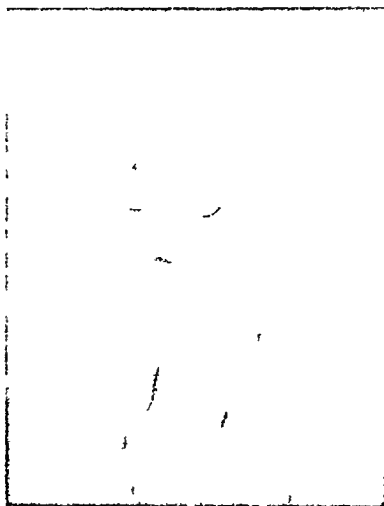
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ON TO DETROIT—NINTH ANNUAL CONVENTION

American Society of Clinical Pathologists

THE Ninth Annual Convention of the American Society of Clinical Pathologists will be held in Detroit, Michigan June 20, 21, and 23, 1930, with headquarters in the Book Cadillac Hotel. The Scientific and Business Sessions will take place in the Crystal Ball Room of the hotel and the Scientific and Commercial Exhibits in the adjoining Italian Garden.

The largest attendance in the history of the A S C P is anticipated. Detroit has been called the ideal convention city. Situated in the heart of the famous Great Lakes district it possesses all of the advantages of an industrially active world center together with the geographical characteristics of a summer resort. Detroit has a population of 1,790,000 persons and is so fortunately located that 70 per cent of the people of the United States are within an overnight's journey. The most important railroads of the nation run crack trains to Detroit; the finest steamers on the Great Lakes offer cool comfortable trips from Cleveland, Buffalo, Duluth, Chicago and intermediate points; bus lines from every important city and established airplane lines furnish the best in speedy travel, and super highways that are without equal make motoring to Detroit a never to be forgotten event. Detroit is a study



DR KENNETH M LANCH
Charleston, South Carolina
President Elect



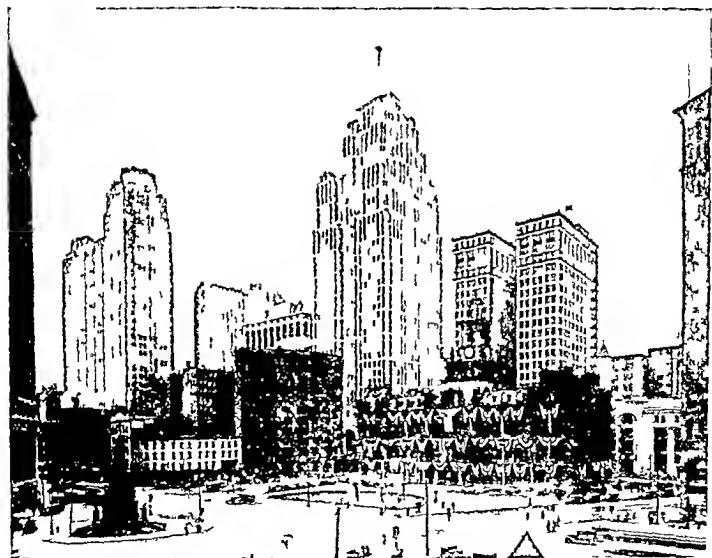
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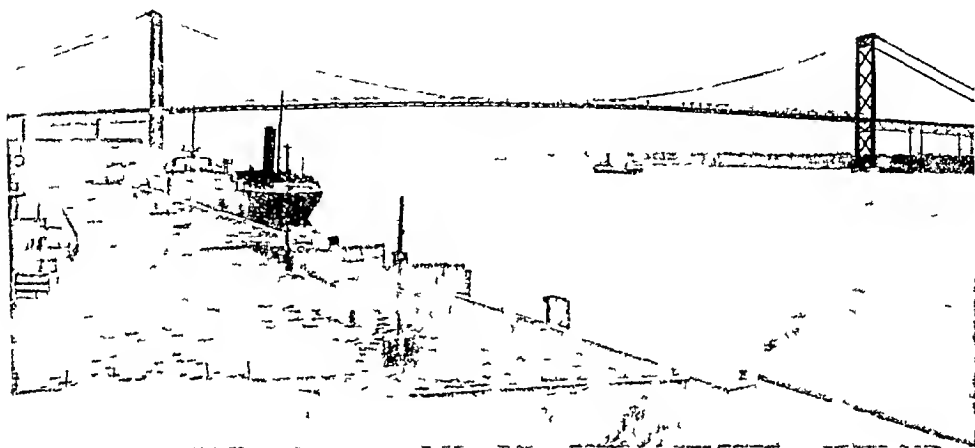


DETROIT SKY LINE FROM THE RIVER

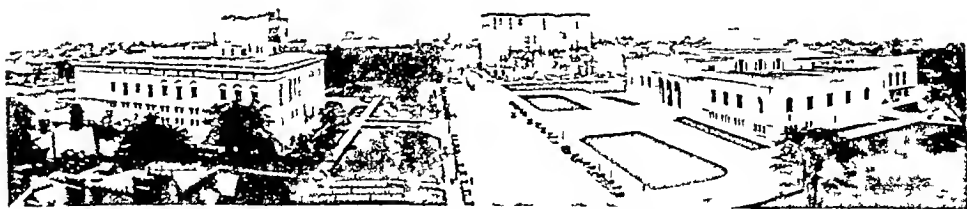


CADILLAC SQUARE

in paradoxes in that its artistic side is of as much importance as its industrial angle. One of the finest art centers in the nation has been developed in the city which emphasizes all of the divisions of the fine arts and offers opportunities to convention delegates and visitors for the study of some of the world's most beautiful paintings and pieces of sculpture. Canada is but a ten-minute ferry ride from Detroit. Windsor, across the river, is a place that



AMBASSADOR BRIDGE

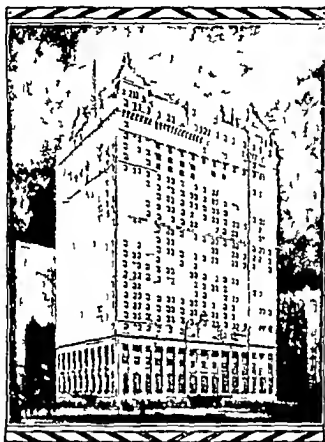


DETROIT'S ART CENTER SHOWING DETROIT PUBLIC LIBRARY AT LEFT AND THE DETROIT INSTITUTE OF ARTS AT THE RIGHT (WOODWARD AVENUE CENTER)

everyone wants to visit. There one can stand on King George's territory and enjoy for a few hours the thrill of being in a foreign land. You will find there the spirit of Britain just as it is in London. The customs, the shops, the speech are a fragment of Piccadilly.

The Scientific Program is full to the brim with excellent papers. A wide variety of subjects should insure the enjoyment of all clinical pathologists no matter what their special genre is. The number of Scientific Exhibitors is greatly increased this year. A gold and a silver medal are offered as first and second award for the best scientific exhibits at the Convention. The Research Committee has planned a Symposium on Agranulocytosis which is expected

to arouse considerable general discussion. The Round Table Discussion on Friday evening ever an enjoyable occasion, contains three pertinent subjects which will no doubt elicit the airing of opinions from all sections of the country. Presentation of medals will take place at the Annual Banquet on Saturday evening at which time we will also receive messages from the American College of Surgeons and the American Medical Association. The Wild Burdick Research Award will be presented to the winner chosen by the Research Committee by President J. H. Black. Dr. Black will also award the two Scientific Exhibits Medals to the recipients selected by the Scientific Exhibits Committee.



BOOK CADILLAC HOTEL

Pule, Davis and Company of Detroit has invited the Fellows of the American Society of Clinical Pathologists in attendance at the convention to visit their laboratories at 1:15 P. M. on Friday and will serve luncheon to them at 2:15. There will be seen demonstrations of certain laboratory procedures and it is expected that this feature of our Convention will be very instructive and interesting.

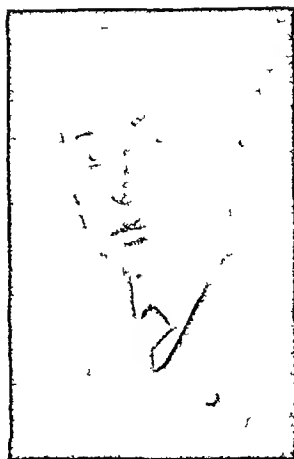
The management of the Book Cadillac Hotel has been very cooperative and assures us the best accommodations available. Members wishing reservations should communicate with the Secretary at once since all space is rapidly being taken up for the meeting of the American Medical Association.

You are therefore urged to lay aside the microscope long enough to attend this coming Convention which from all indications promises to be our best ever.

ON TO DETROIT!



DR. FRANK W. HARTMAN
 Detroit, Michigan
 Chairman, Executive Committee



DR. A. H. SANFORD
 Rochester, Minnesota
 Chairman, Necrology Committee
 Member, Executive Committee



DR. FREDERICK E. SONDEN
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DR. JOHN A. KOLMER
 Philadelphia, Pa.
 Chairman, Publication Committee
 Member, Executive Committee

American Society of Clinical Pathologists

Program

Ninth Annual Convention

Detroit Michigan

June 20 21 and 23 1930

FRIDAY MORNING JUNE 20 8 AM

Short Business Session

Scientific Program

The Diagnosis of Pregnancy by the Demonstration of the Hormone of the Anterior Lobe of the Hypophysis in the Urine By H I Reinhart MD and Ernest Scott MD Columbus Ohio

Studies on Von Ehillung Count By Asher Laguda MD Newark New Jersey

Direct Calculation of the Volume and Hemoglobin Content of the Erythrocyte A Comparison with Color Index Volume Index and Saturation Index Determinations By M M Wintrobe MD New Orleans Louisiana (By invitation)

The Kline Precipitation Reaction as a Routine Adjunct to the Complement Fixation Test in the Serological Study of Syphilis By Robert A Kalluske MD Atlantic City New Jersey

Unexpected Finding in Unexpected Deaths By Wm J Deadman MB Hamilton Canada
Restraining the Unruly Patient while Performing Spinal Puncture Motion Pictures Demonstration By H A Heise MD Uniontown Pennsylvania

The Reaction of the Meninges to Therapeutic Serum By Wm M Shippey MD Wheeling West Virginia

Coronary Occlusion By Ernest Scott MD and Mary K Holz Columbus Ohio Title read by title

FRIDAY AFTERNOON, JUNE 20

Visit to Laboratories of Parke Davis and Company Laboratory Demonstration 1 to 3 PM
Luncheon 3 to 4 PM

Symposium on Agranulocytosis

Hematological Aspects of Agranulocytosis and Other Disease Accompanied by Extreme Leucopenia By Nathan Rosenthal MD New York NY

Agranulocytic Syndromes By E T Miloslavich MD and Francis D Murphy MD Milwaukee Wisconsin

Report of Case with Recovery Lantern Slides By L W Tarron MD Bismarck North Dakota

Gastric Manifestations of Leukemia Leukemia By Kuno Ikeda MD St Paul Minnesota

FRIDAY EVENING JUNE 20 7 to 9 PM

Round Table Discussion

Development of an Autopsy Service By Asher Laguda MD Newark New Jersey

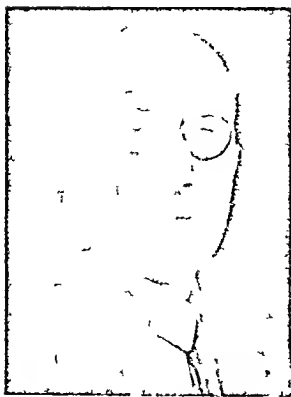
The Development of Local Pathological Societies as Component Parts of the American Society of Clinical Pathologists By Wm M Shippey MD Wheeling West Virginia
Is It Ethical for a Clinical Pathologist to Advertise? By J J Moore MD Chicago Illinois

SATURDAY MORNING, JUNE 21 9 AM

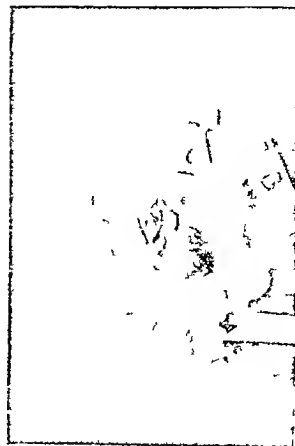
Hemorrhage with Sudden Death in Trichobronchial Lymph Node Tuberculosis in Adults By H A Callis MD Tuskegee Alabama

Additional Observations on Isolating Tubercle Bacilli By H J Corger MD and Nao Uvei PhD Denver Colorado

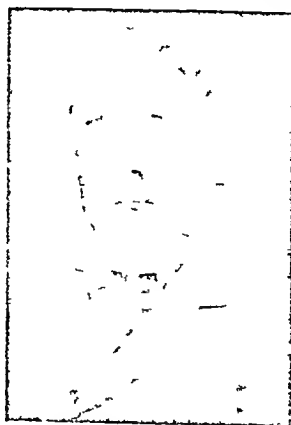
The Use of the Photo Electric Effect in Clinical Pathology By Wm G Exton MD Newark New Jersey



DR. WILLIAM G. EXTON
Newark, N. J.
Member, Executive Committee



DR. ALFRED S. GIORDANO
South Bend, Indiana
Member, Executive Committee



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DR. REUBEN OTTENBERG
New York City
Member, Board of Censors



DR. ERNEST SCOTT
Columbus, Ohio
Member, Board of Censors

- The Electromotive Thermometer An Instrument Equipped with Thermocouples for the Measurement of Skin and Intramuscular Temperatures By Charles Sheard, Ph D, Rochester, Minnesota
- Methods and Effects of Increasing the Urinary Constituents in the Body By Frank W Hartman, M.D., Detroit, Michigan
- Experimental Fat Necrosis in Various Vertebrates By M Pinson Neal M.D., and Max M Ellis, Ph D, Columbia, Missouri
- Vascular Injury by the Pneumococcus and Its Relation to Red Hepatization in Lobular Pneumonia By Theodore J Curphey, M.D New York, N Y
- Gas Bacillus Infection in Civil Life By Walter E King, M.D., Detroit, Michigan

SATURDAY AFTERNOON, JUNE 21 2 P.M.

- Gastrointestinal Disturbances in Endocrine Diseases By Michael G Wohl, M.D., Philadelphia, Pa
- Pathogenesis of Goitre By B Markowitz, M.D Bloomington Illinois
- Thyroid—The Inflammatory Nature of Nodular Goitre as a Chronic Thyroiditis By Robert A. Kelly, M.D., Washington D C
- The Present Status of Our Knowledge of Cancer By William Carpenter MacCarthy M.D., Rochester, Minnesota
- Stains in the Microscopic Diagnosis of Malignancy By Charles Geschickter M.D., Baltimore, Maryland (By invitation)
- The Diagnosis of Border Line Breast Tumors By Joseph C Bloodgood, M.D Baltimore Maryland (By invitation)
- Some Unusual Pathologic Tissues By Philip Hilkowitz M.D Denver Colorado

SATURDAY EVENING, JUNE 21, 7.30 P.M.

Annual Banquet

- Presidential Address By J H Black M.D Dallas, Texas
- Measuring the Efficiency of the Clinical Laboratory By Malcolm T MacEachern, M.D., Associate Director of the American College of Surgeons Chicago Illinois
- Clinical Laboratories and Medical Progress By N P Colwell, M.D Secretary of the Council on Medical Education and Hospitals of the American Medical Association, Chicago Illinois
- Presentation of Scientific Exhibits Medals By President J H Black, M.D
- Presentation of the Ward Burdick Research Award By President J H Black, M.D

MONDAY, JUNE 23

Business Session

- Call to order
- Reading of Minutes
- Unfinished Business
- Reports of Committees

Executive Committee—Frank W Hartman M.D Detroit Michigan, Chairman

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Necrology Committee—A H Sanford M.D, Rochester Minnesota, Chairman

- Report of the Board of Censors—Election of New Members

F H Lamb, M.D Davenport, Iowa, Chairman

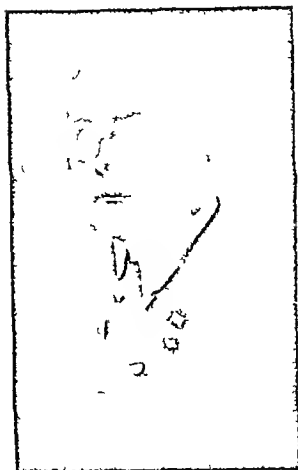
- New Business

- Report of the Nominating Committee—Nomination of Officers

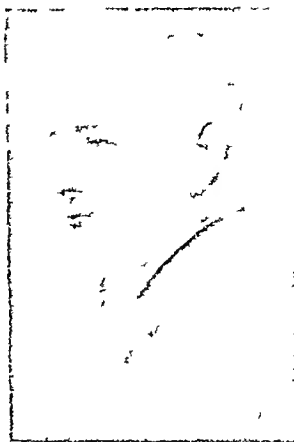
- Election of Officers

- Induction of Officers

- Adjournment



DR. B. W. RHAVY
Fort Wayne, Indiana
Member, Board of Censors



DR. WARREN T. VAUGHAN
Richmond, Virginia
Member, Board of Censors



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Washington D. C.
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Battle Creek Michigan
Chairman Program Com-
mittee



DR F B JOHNSON
Charleston S C
Chairman Public Relations
Committee



DR CLARENCE I OWEN
Detroit Michigan
Chairman Scientific Exh-
bits Committee

Scientific Exhibits

An Exhibit of Preserved Specimens and Photographs Illustrating the Changes Produced in the Ovaries of Experimental Animals as the Result of the Injection of Urine Containing Pituitary Hormone By Ernest Scott, MD, Columbus, Ohio

Microscopic Slide Precipitation Tests for Syphilis of Unheated Serum, Heated Serum, Defibrinated Finger Blood and Unconcentrated Spinal Fluid By B S Khne, MD, Cleveland, Ohio

Photographs and Microphotographs Illustrating Chronic Thyroiditis By Robert A Keilly MD, Washington, D C

The Aschheim Zondek Pregnancy Test By E R Murgage MD, and Rodney H Jones, MD, Denver, Colorado

Coccidioidal Granuloma By W T Cummins, MD, San Francisco, California, Joseph K Smith, MD, Bakersfield, California and C H Halliday, Baltimore, Maryland

Experimental Pneumococcus Infection in the Horse By Theodore J Curphew, MD New York, N Y

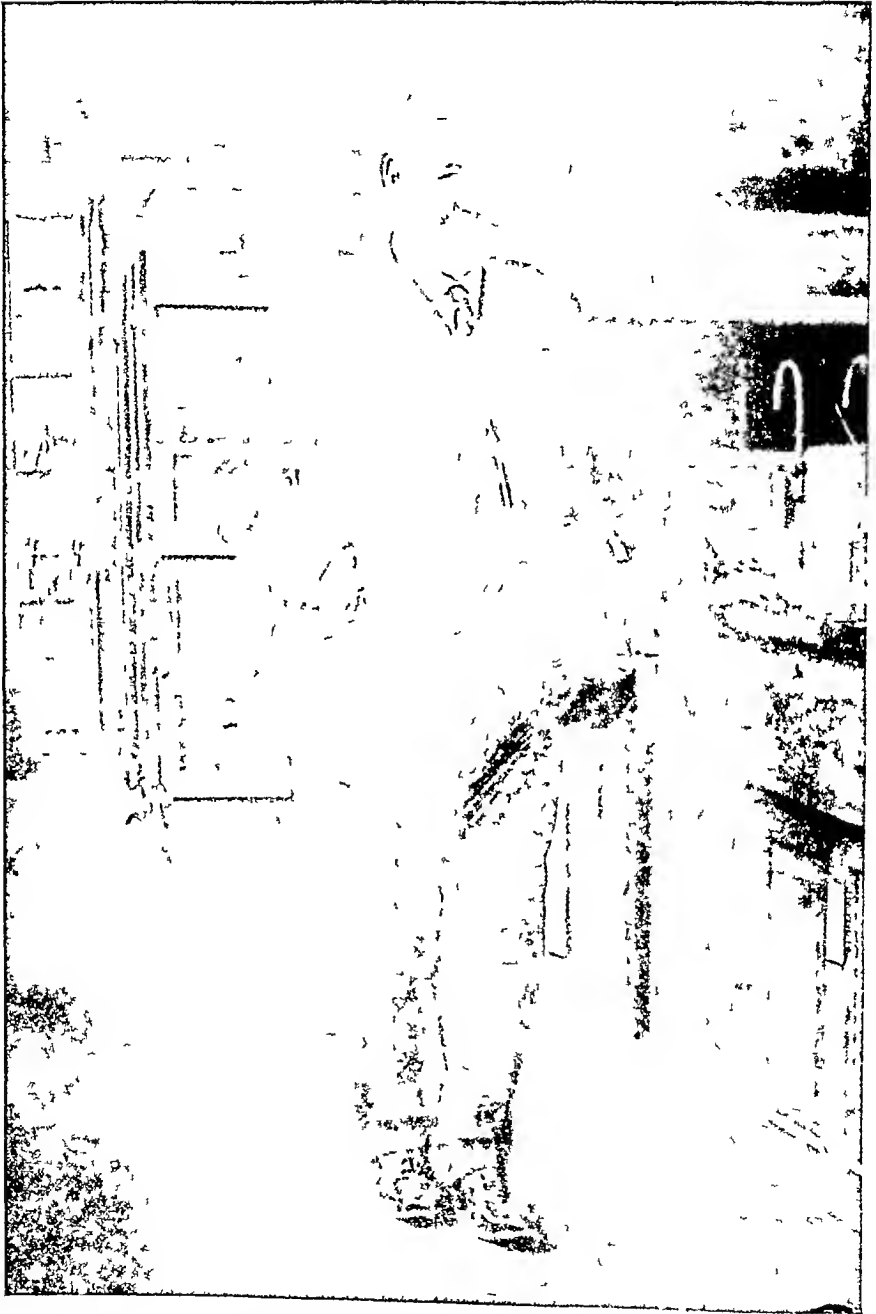
Commercial Exhibits

Carl Zeiss, Inc, 485 Fifth Avenue, New York, N Y

LaMotte Chemical Products Company, McCormick Building, Baltimore Maryland

E Leitz, Inc, 60 E 10th Street, New York, N Y

Central Scientific Company, 460 East Ohio Street, Chicago, Illinois



DR VAUGHAN IN HIS LABORATORY, 1914

The Publisher
and Editorial Associates,
Past and Present
of The Journal of Laboratory and Clinical Medicine
Dedicate with Deep Affection
This Number
To the Memory of its First
Editor in-chief

VICTOR CLARENCE VAUGHAN

To Whose Initiative, Enthusiasm and Untiring Zeal
The Journal
Owes its Present Prestige and Usefulness
As a Medium
For the Exchange of Thought and Experience
Among
Physicians and Scientists

It may not be given to us to solve the
riddle of the universe but this need not
deter us from doing the duty that lies
so plainly before us and the most exalted
privilege that comes to man is to labor
for the uplift of his race

—*Victor C Vaughan*

The Journal of Laboratory and Clinical Medicine

VOL. XV

ST. LOUIS, MO., JUNE, 1930

No. 9

VICTOR CLARENCE VAUGHAN*

PHYSICIAN, educator, biochemist, hygienist, patriot Born at Mount Airy, Randolph County, Missouri, October 27, 1851, died at his home in Richmond Virginia November 21, 1929

Victor Vaughan first emerged from the obscurity of youth and adolescence at the age of nineteen as professor of Latin at Mount Pleasant College, Huntsville, Missouri In his earlier years he had been tutored by a man whose first love was Latin and who so drilled this dead language into the young boy that the two of them were able and accustomed to carry on their daily conversations in Latin It was but natural then, that immediately upon his graduation from Mount Pleasant College, he should devote his energies to that subject in which he was most proficient But for a man whose subsequent life has proved him to be an indefatigable searcher for the truth in the great unknown realms of science the teaching of a dead language could scarcely be expected to become his permanent metier

He discovered the latter quite by accident In an unused room in Mount Pleasant College which had been closed through the Civil War, Victor Vaughan discovered a number of unopened packing cases which on investigation proved to contain a complete outfit for a chemical laboratory Obtaining permission to set up the laboratory, and to experiment with the various chemicals, he soon became fascinated with the work and within a short time was teaching chemistry along with his Latin Throughout the remainder of his life the viewpoint of the chemist dominated his contributions to research

In 1874 he entered the University of Michigan to pursue his chemical studies, and a year later added the degree of M.S. to that of B.S. obtained in Missouri In 1876 he received the degree of Doctor of Philosophy and two years later that of Doctor of Medicine In 1897 he was made an honorary Doctor of Science by the University of Western Pennsylvania Four times he received the honor of the degree of Doctor of Laws, the highest honor

*Reprinted with permission from the Americana Annual of the Encyclopedia Americana 1930

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deter us from doing the duty that lies
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—Victor C. Vaughan

before, and Robert Koch had demonstrated that the anthrax bacillus is the cause of anthrax, the birth of the science of bacteriology dates from 1882, when Koch developed methods for isolating bacteria. In 1887 when the hygienic laboratory was being built, bacteriology was but five years old and there were no trained bacteriologists in this country. In 1888 Dr. Vaughan therefore spent some time in the laboratory of Dr. Robert Koch in Berlin, gaining an understanding and mastery of this new science. Immediately upon his return this work was introduced into the hygienic laboratory. This was the first laboratory in the United States offering systematic teaching of bacteriology to students and physicians, and the second in the world.

As a consequence of his rapidly developing preeminence in the fields of hygiene and sanitation and toxicology, Dr. Vaughan was offered in the nineties the professorship of hygiene at Bellevue Medical College, New York, together with the position of coroner for the city of New York. These honors, however, he declined, feeling that he could never be happy living in the great city. At about this time he was made Dean of the Department of Medicine and Surgery at the University of Michigan, a position which he held for thirty years, until his retirement in 1921. Under his direction the school grew steadily until it became one of the greatest medical schools in the United States. He gathered around himself a faculty of the best minds in medicine, and as rapidly as another great medical school would take away individual members for its own faculty, he would replace them with other men of equal brilliance.

From the time of his tutelage under Robert Koch, Dr. Vaughan's first interest in the research laboratory remained always the mechanism by which bacteria cause disease and the manner in which the living body combats bacterial infection. This was but a logical step from the study of the chemical causation of disease, and throughout the remainder of his work his viewpoint even in the field of bacteriology was that of a chemist. This is in evidence even in the last essay which he wrote, *A Chemical Concept of the Origin and Development of Life*, in 1927. Dr. Vaughan has with entire justice been called one of the founders in this country of the modern science of biochemistry or the chemistry of life.

His contributions to medical literature are too numerous to detail, being represented by about two hundred and fifty short articles and seventeen books, some of which have gone through several editions. Among the outstanding contributions emanating from his laboratory may be mentioned *The Michigan Method of Water Analysis*, the discovery of tyrotoxin or cheese poisoning, the demonstration that a variety of bacteria may be responsible for the summer diarrheas of infancy, the discovery that nuclein, a constituent of normal blood, possesses germicidal properties, and chemical researches into the nature of bacteria and their reaction with living tissues which culminated in Vaughan's theory of infection and immunity, a theory which today still colors most of our understanding of these processes.

In 1898 Dr. Vaughan joined the Thirty Third Michigan Volunteer Infantry as major and surgeon and served through the Spanish American War as division surgeon. He was the first surgeon to come under the fire of the Spanish batteries at the Battle of Santiago. While in Cuba he contracted

that can be conferred by an institution of higher learning. He received this degree from the University of Michigan in 1900, Central College, Missouri, in 1910, Jefferson Medical College, Philadelphia, in 1915, and from the University of Missouri in 1923. An unusual honor was the conferring on him of the honorary degree of Doctor of Medicine by the University of Illinois in 1894.

Dr. Vaughan's first contribution from the chemical laboratories of the University of Michigan, appearing in 1875, was on the separation of arsenic from other metals. Throughout the succeeding fifty years the study of organic and inorganic poisons held greatest interest for him, and his contributions to the subject have been authoritative. Before long he was recognized as one of the leading toxicologists of the country, and his services were in constant demand in cases of medico-legal dispute.

As early as 1875 Dr. Vaughan became associated with the Medical School of the University of Michigan as instructor in medical chemistry. In 1878 he published his second book, a textbook of physiologic chemistry which went through three editions in as many years. In 1880 he was made assistant professor, and in 1883 professor of physiologic and pathologic chemistry and associate professor of therapeutics and materia medica in the Medical School of the University of Michigan.

During these years Dr. Vaughan's research work paralleled quite closely the line of his teaching work. He continued always interested primarily in the chemical causes of disease and the changes in the human body consequent on contact with these causes.

In 1880 he became interested in the contamination of drinking water, and this eventually led him into the field of public health. The inadequate and unsanitary facilities for obtaining drinking water at that time rendered the question of pollution most important. In those days the only method of examination was chemical, and gradually the function of examining water supplies from all over the state of Michigan fell to Dr. Vaughan. He soon saw that this was but a very small portion of the general problem of sanitation and his interest in this field gradually broadened. In 1886 he wrote the Lomax prize essay of the American Public Health Association entitled *Healthy Homes and Foods for the Working Classes*, which went through many editions and was translated into most of the modern languages. In 1883 he was appointed a member of the Michigan State Board of Health, and for the following thirty-six years he served as its president, until 1919 when the board was disbanded and replaced by a commissioner of health.

During his first fifteen years at Ann Arbor, Dr. Vaughan carried on his investigations in the chemical laboratory. However, it gradually became apparent that the old chemical laboratory was inadequate for pursuing problems relating to health and disease. He therefore sought and obtained a grant from the Michigan legislature in 1887 with which to build and equip a hygienic laboratory at the University. At the same time he was made professor of hygiene and physiological chemistry and director of the hygienic laboratory. These positions he retained until his retirement from the university.

At this time the newly developing science of bacteriology was the great advance of the day. Although Pasteur had laid the foundations some years

Following the Spanish American War he received a citation for gallantry under fire, and following the World War he received the Distinguished Service Medal for his work in epidemiology, and was made a Knight of the Legion of Honor by the French government.

In 1928 he received the Kober Medal of the Association of American Physicians for outstanding contributions to his profession.

No biographic review of Dr. Vaughan's rich and varied life would be complete without some mention of his first twenty years as a physician, during which he devoted half of his time to the practice of medicine. It was what he saw and heard at the sick bed that filled him with a compassion and sympathy which lasted throughout his life, which won for him devoted friends and admirers throughout the world, and which spurred him on throughout his life in his battle against filth, poverty, and ignorance, against typhoid fever and tuberculosis and against the germs of disease. Unlike many of the present day research workers, he had seen on both sides of the curtain, he was able to work not only as a laboratory investigator interested in an abstract problem but also with the zeal of one who had witnessed human suffering and who hoped to do his bit toward ameliorating it. Fortunately, he has left in *A Doctor's Memories* a delightful narrative of his unique experiences and of his contacts with the great minds of his time, both in Europe and America.

In 1927, upon his return from the Orient where he had attended at Tokio a meeting of the Pan Pacific Congress as a representative of the government of the United States, the National Research Council, and the American Medical Association, he suffered a mild apoplectic seizure from which he recovered, but which terminated his participation in active work.

—Warren T. Vaughan

yellow fever, and after a prolonged convalescence, he was invalided home to Washington. Here he, Major Walter Reed, and Major Edward Shakespeare were appointed as a commission to study typhoid fever among the Spanish War troops. This committee made revolutionary observations on the nature and manner of the spread of typhoid. Reed and Shakespeare having died, it fell upon Major Vaughan to complete the work and write the report. Thus he did in a massive two volume contribution which stands today as one of the most authoritative works on the epidemiology of typhoid and kindred diseases.

From the time of the Spanish-American War until his retirement, Dr Vaughan remained in close contact with the army and the United States Public Health Service, serving on the advisory board of the Surgeon-General of the United States Public Health Service, and on the advisory board of the Hygienic Laboratory at Washington.

In September, 1916, many months before the entrance of the United States into the World War, the National Research Council was organized as a committee from the National Academy of Science, to serve the president of the United States in an advisory capacity in preparation for war. Dr Vaughan, who was one of the organizers of this committee, served with it throughout the war, and following its reorganization after peace, became the first chairman of the medical division of the National Research Council. This position, requiring full time, necessitated Dr Vaughan severing his connection with the medical school of the University of Michigan and moving to Washington. Throughout the World War he served as colonel in charge of the Division of Communicable Diseases in the Surgeon-General's office, and on the executive committee of the general medical board of the Council of National Defense. By virtue of his position in the army, he was able to apply his wide knowledge of hygiene, sanitation, and epidemiology in the organization and supervision of the army training camps throughout the United States. Following the close of hostilities, he wrote a two-volume treatise on *Epidemiology and Public Health*, into which he has incorporated his own vast experience together with the experience of others.

As an editor he founded the *Physician and Surgeon* in 1888, the *JOURNAL OF LABORATORY AND CLINICAL MEDICINE*, in 1915, and in 1923 served as the first editor of *Hygeia*, a popular journal published by the American Medical Association.

Dr Vaughan served as president of the American Medical Association in 1914-15, and for many years remained a member of its Council on Medical Education. From 1919 to 1923 he was chairman of its Council on Health and Public Instruction. He was a member of the Association of American Physicians, being its president in 1908-9. He was president of the American Tuberculosis Association in 1919. He was also a member of the National Academy of Sciences, the American Philosophical Society, the French Society of Hygiene, the Hungarian Society of Hygiene, and other learned societies.

He served for several years on the board of directors of the International Health Board, which has done more than any other one organization to stamp out hookworm, malaria, and yellow fever from the Americas.

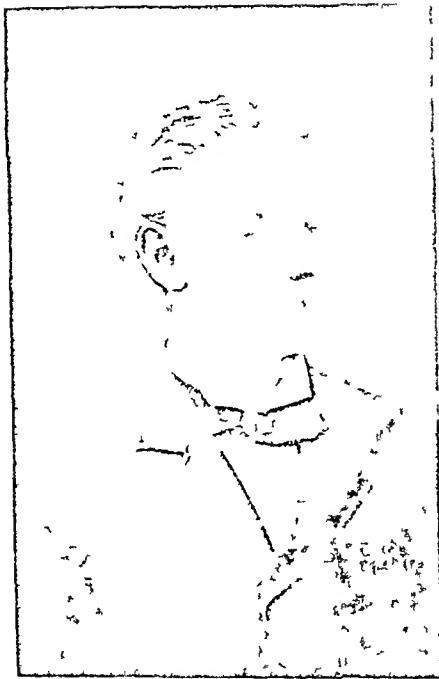
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—Warren T. Vaughan



VICTOR C VAUGHAN, PH D
Instructor in Urinary Analysis, 1877

A GREAT PIONEER PASSES*

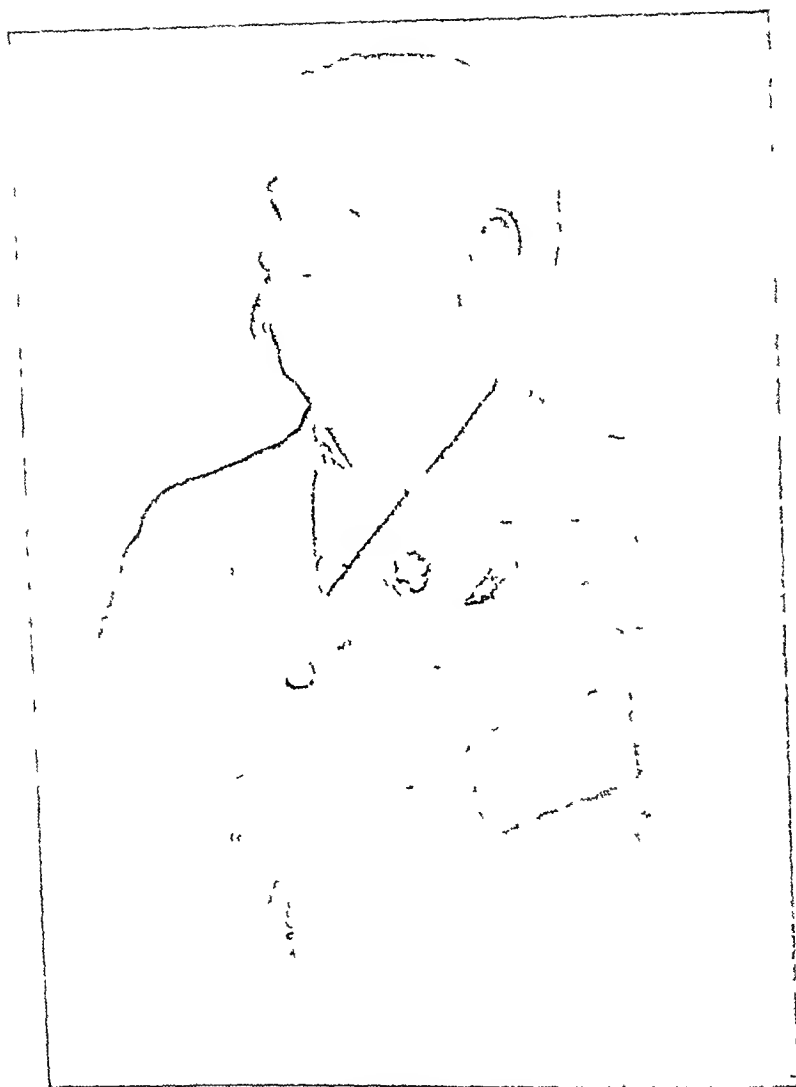
AMERICAN science today mourns the passing of a great pioneer. When Dr Victor Vaughan began scientific research, there were thousands of surgeons who thought Listerism a fad, and other thousands who doubted the whole germ theory enunciated by Pasteur. In medical education, standards were so low that a young man could register at almost any medical college if he could read and write, and he could "qualify" for his profession, with the title of doctor of medicine, after two years of training.

It was Dr Vaughan's extraordinary fortune to contribute both to the new science and to the new medical education. At Ann Arbor he inaugurated the system of premedical training that has since been generally adopted. He insisted, also, that his students equip themselves with a background of cultural study, especially in languages. He built up the medical faculty of the University of Michigan on the solid foundation of brains devoting all of his appropriations to the employment of outstanding men in their professions. He was shrewd enough to reason that if he brought great teachers to the school, the graduates would quickly see to it that adequate equipment was provided by the state.

In his own scientific work, Dr Vaughan systematized the developing principles of epidemiology and helped create in this country the new science of physiological chemistry, which has now become biochemistry. His influence was as important as his discoveries. He sent out well equipped investigators, a multitude of them, and he was in full fellowship with all that great company of scientists whose joint work in sanitation and hygiene has transformed American public health within the last thirty years.

Dr Vaughan grew old as a scientist should—gradually abandoning research, but never losing interest turning happily to past memories without permitting past achievements to overshadow recent advances. His *A Doctor's Memories* was a charming book worthy to stand on the same shelf with such modern medical classics of biography as Wyeth's autobiography, Vallery Radot's *Pasteur*, and Cushing's *Osler*. His lovable personality and his rare skill as a raconteur made it certain that wherever he sat was the head of the table. Brief as was his residence here in Richmond he had the admiration of all who are wise enough to see in great scientists the prophets of a nobler happier social order.

*An editorial in the Richmond (Virginia) *News Leader* November 22 1929



AT BUDAPEST, 1914

VAUGHAN, THE MAN*

THE name of Vaughan, like that of Angell, has become a synonym for beloved traits of character, in this town

They are traditions

Great men were those two Great in the sense that they accomplished much and became famous through their endeavors but even greater because in spite of those attainments their personalities were supremely human There was a magic warmth in their very presence

Angell joined the immortals long ago Vaughan laid down his burdens Thursday night in Virginia

The former Michigan medical dean was nearly eighty years of age It was a strenuous life he led one that might have been calculated to shorten his span It was strenuous in the sense that he was forever busy, rising at an early hour putting in a day of work that was exceedingly full But there was no nervous waste of energy

Vaughan accomplished things quietly, without apparent strain There was no lost motion, he had the capacity to do things without unnecessary exertion Maybe that is one reason he lived so long

But another reason must have been his human qualities He could always take out time for communion with his friends We have an idea that he may have been spared so long because he was so much beloved

It may be fine to enjoy international renown as a scientist It must be gratifying to have such accomplishments as those of Vaughan to one's credit But in his *Memories* human beings and human relationships predominated That is significant It confirms the belief that his scientific brain capacious as it undoubtedly was could not compare in size with his human heart In deed, it was through love of humanity that he was devoted to science

That heart beat for the University and for Ann Arbor He had watched them both grow, was identified with that growth and he loved their pasts yet he was proud of the progress here both on and off the campus Sentiment must not be permitted to interfere with progress he told the writer not long ago, while discussing the changes here He could take delight in the past while taking pride in the future

Though he died the other day in Virginia he still lives in Ann Arbor He, like Angell is one of the town's immortals

The spirits of them both are living forces of inspiration

Not because of what they did but because they were the kind of men they were

An editorial in the Ann Arbor (Michigan) Daily News November 23 1919



THE ANN ARBOR HOME

STANDARD BEARER

IN THE death of Dr Victor C. Vaughan the medical profession lost one of its greatest teachers, and the public lost a friend who worked incessantly for the cure and prevention of disease.

As one of Dr. Vaughan's students in the early days I perhaps can give a picture of the man, his ambitions, his influence as a teacher, and those personal attributes that so endeared him to his students and to all who came in contact with him.

Dr. Vaughan was a comparatively young man when he came to the Medical Department of the University of Michigan. He had been in Ann Arbor only a few years when I entered the medical school as a student. The chair of medicine was held by Dr. Alonzo B. Palmer. At that time Dr. Palmer was eighty years of age and quite naturally it was Dr. Vaughan who carried the load of giving the students a modern view of medicine. Dr. Vaughan's outlook on medicine was a generation ahead of his time. He was one of the first to realize the influence that physics and chemistry were to have on medicine and his students were well prepared for the great changes in the science and art of medicine which have come about in the present day. He aroused in his students an enthusiasm and a thirst for knowledge and beyond all he inspired them with an interest in the tomorrow of medicine which I may say, was the mainspring of his life.

Whereas Dr. Vaughan might have taken up clinical medicine and made a great success in its practice, he chose to devote himself to preventive medicine. In the clinical work which he did undertake he showed great sagacity and the acumen of a fine practitioner of medicine. Although he was interested in the individual in what might be called retail medicine, his heart was in the wholesale problems which affected the mass of the people.

Researches into public welfare always have been carried on by men like Dr. Vaughan, and too rarely do the people understand the significance of the work sufficiently well to give the reward where it is due. Fortunately Dr. Vaughan's name and fame do not depend on popular acclaim. He lives rather in the minds and hearts of the medical profession. The members of this profession gave him the greatest gift within their power, the presidency of the American Medical Association. They also appointed him to responsible positions which had to do with the solution of problems of public health and the adaptation of health measures to the needs of the people.

The value of Dr. Vaughan's work during the World War cannot be overestimated. Owing to the inadequate sanitation of the hastily constructed military camps and the hurriedly mobilized medical personnel untrained for their duties in military service epidemics of disease were appalling. Dr. Vaughan in the office of the surgeon general rendered invaluable service in the work of stemming disease and bringing about better organization of the medical personnel and of the newly enlisted men in camps. Many times I heard Surgeon

General Goigas speak gratefully of Dr. Vaughan and his associates who so willingly and wholeheartedly gave their time and effort to support the work in the office of the surgeon general.

I came to know Dr. Vaughan well in those years in medical school. I knew him in his home, in his laboratory, and in his contacts with the sick, and I had the pleasure and profit of close association with him during all the years that followed. It may seem strange that a surgeon and a medical scientist should have had so much in common, but the friendship which was engendered in that early day continued through life. His viewpoints were most stimulating, and his friendly interest matched my admiration for him and his work.

In penning these few words concerning my old teacher and friend, I wish to acknowledge the influence he had on my professional life and which he had on the lives of hundreds of medical students in the years that have passed between. He not only helped the members of the medical profession to a keener sense of their professional responsibilities, to the individual patient and to sick human beings collectively, but he induced them to live up to a standard of ethics which he himself followed all his life.

—W. J. Mayo,
Rochester, Minn.

VICTOR CLARENCE VAUGHAN AND THE UNIVERSITY *

WE HAVE assembled today to commemorate the passing of a man whom it is altogether fitting we should honor—a man whom we knew and loved as a friend, and admired and respected as a colleague.

While as permanent, it is to be hoped as any human institution and thus greater than any individual, the University is nevertheless molded by the strong and able men of broad vision on its faculties. It is doubtful if the impresses of the personalities of these men upon their institution will ever be erased and certainly it is earnestly to be hoped that they are indelible.

Who can doubt that Dr Victor Clarence Vaughan, during the period of his active service at the University of Michigan was a powerful factor for good in shaping its destinies? Others will today discuss his contributions to science and to the practice of medicine, and his relations with his colleagues. The high ideals of scholarship, the research ability and the cooperative spirit which were his are all attributes of the successful professor.

In addition to these however Dr Vaughan had to an enviable degree the characteristics which made him an administrator who could not only lead his school according to the best contemporary standards but who could also anticipate future conditions and academic requirements in his chosen field all without failing to observe the proper perspective of his unit to the parent institution. Thus it was that he was willing to give fully of his time and strength to the founding of the Graduate School, the Senate Council, the Board in Control of Athletics and such organizations as the Scientific Club and the Research Club, and to the assistance of the Alumni Association. In short, while devoted to the development of the medical units, it was ever his governing conception that these units should not be built at the expense of other University departments, but that the University as a whole should be steadily developed to maintain the proper relation between the institution and an ever better school of medicine.

The University recognized Dr Vaughan's ideals and ability in the way he would have preferred—by giving him constantly increasing responsibilities. It was characteristic of the man that he accepted these responsibilities and labored diligently for the general good of the University and for the development of the Medical School, without neglecting to serve ably his community, state and nation and to establish and maintain an enviable record as a teacher and investigator. His service was therefore the maximum one that a member of the staff can render and we need not point out in detail the evidence of his success as a university officer. Dr Vaughan's monument is a Medical School.

Memorial Meeting for Doctor Victor C. Vaughan at the Lybia Mendelssohn Theatre
Ann Arbor December 3, 1929

Courtesy of Wilfred B. Shaw, Director of Alumni Relations, University of Michigan

which has few rivals for its output of scientific contributions and men of marked ability as scholars and practitioners, and the other flourishing departments which are in large measure the materialization of his dreams. We may pause to observe that the University as a whole is a better institution than it would have been without his assistance.

I have said in another place that the University is in a very real sense its faculty. "Buildings are needed, equipment is required, and funds for current expenses must be provided, but since these material requirements are worthless without men to use them, the effectiveness of the University may be said to be determined by the men who compose its faculties." In Dr. Vaughan's life we will have before us always a standard of merit for the appraisement of our value to the institution that we serve.

—*Alexander G. Ruthven,*
Ann Arbor, Mich.

REMARKS ON BEHALF OF THE BOARD OF REGENTS*

THERE is but one measure of greatness—the contribution that one makes to world betterment. To few is it given in both ability and opportunity to reach the summit, and merit the supreme reward. What the purpose of our living and striving may be is beyond our ken. It is sufficient that we play our part in the drama of progress trusting to a wise Dictator of all things.

By this standard and conception, our beloved and departed friend has won a place in the seats of the mighty.

It was my good fortune to have known Dr. Vaughan as a teacher in my student days, and as an affectionate helpful friend. He was a great inspiration to higher ideals—a leader and an originator.

Prophylactic medicine owes to him an immense debt. He early recognized the value of preventive measures and strove with all his rare talents to make them effective for mankind.

During his active life no great advance was made to which he was not an important contributor. The pioneer always travels an uphill road but, true to his convictions, he fought the battle never daunted or discouraged. Fortunately he lived long enough to see many of his dreams come true and his judgments justified. This must have been a blessing in his declining years.

He early conceived the value of research and progress in the fundamental sciences and surrounded himself with a rare corps of coworkers who were without superiors.

As dean of a medical school situated in a small city where in those days, variety of clinical material was wanting, he found it expedient and wise to build up and emphasize the laboratory or science courses in medicine to compensate for the deficiency in the medical arts or clinical courses. This was so well accomplished that he won distinction for his school and gave to it high standing.

No honor which medicine could confer was withheld from him. He was a world character because a world benefactor. And I have often said that a letter addressed to Dr. Victor C. Vaughan, without any other direction, and mailed from any part of the globe would reach him at Ann Arbor.

While, as students we feared his courses we knew that he was exacting of us no more than he demanded of himself, and we respected and loved him. No student of his ever left the University without admiration and affection for him. This must have been a source of much happiness to him.

I had the prized privilege of often being a guest in the home of Dr. and Mrs. Vaughan, where the whole atmosphere was that of culture, peace and beauty. They were blessed with an unusual family of boys and the relationship between father and mother and sons was movingly intimate and sympa-

*Courtesy of Director of Alumni Relations University of Michigan

thetic, they shared a just pride in the achievements of one another, and all interests were mutual and affectionate

As dean and administrator of the Medical School, he had the confidence of the Board of Regents. He possessed that quality which is so large a factor in successful administration—he knew men and could justly appraise their possibilities and futures. We trusted his judgment and always gave to him the full measure of support within our means. He was appreciative of this attitude and relationship and reasonable in his requests.

In 1921 he began to feel that his health would no longer allow him to carry the burden, and that younger shoulders must take the load. It was a difficult conclusion for him and for us, but we were forced to bow to nature's limitations.

He lives immortal in a world made better by his living and has joined the "chorus invisible."

—Walter H. Sawyer,
Hilldale, Mich

DR VAUGHAN'S INFLUENCE IN MEDICAL EDUCATION

DOCTOR VAUGHAN was connected with the University of Michigan for a period of forty six years, a term of service which I believe has been exceeded only once. But this long service is not of itself the reason for thus honoring his memory. Rather, it is because of the high distinction which he attained in his profession, the enduring impress which he made on the Medical School and on the University as a whole. In honoring him the University but honors itself.

Great as was his interest in the scientific work which he conducted, it was no greater than his devotion to the advancement of the Medical School. Fortunate, indeed, was the University that his guiding hand was at the helm in the crucial period which marked the transition from the old to modern medicine. It has been truly said of him that he took a great part in making his *Alma Mater* one of the best known medical schools.

Medical education in this country has passed through a period of evolution from the old apprenticeship system or preceptorship through the proprietary or didactic colleges—which, as A. Flexner once stated, multiplied by fission or by a process of spontaneous generation—to the University schools with scientific discipline as the corner stone.

In passing, it may be mentioned that in the Act of 1817, establishing the University of Michigan, one of the thirteen professorships contemplated was that of Medical Sciences. In the then existing state of knowledge it seemed to the founders that a faculty of one was sufficient to cover the entire field of medicine. There is no evidence that any one functioned in this position, but at least one instance of that kind is known—that of Nathan Smith who established the medical department at Dartmouth and who was himself for twelve years practically its entire faculty.

When the Medical School was opened in 1850 it had a faculty of five of whom Douglas and Sager were men of scientific bent and it was because of the influence of these men that the school early took on a scientific character. Unlike the proprietary schools of that period, the school was from the beginning an integral part of the University. In those days the term of instruction in the medical colleges of the country, almost without exception, extended over two terms of four months each. The new school began with a requirement of two years of six months each. The course of study was extended, in 1877, to two years of nine months and in 1880 to three years which allowed a graded curriculum to be established.

In the decade which followed the experimental sciences were rapidly developing, and it is at this point that Doctor Vaughan's influence began. After the death of Dean Palmer in 1887 Doctor Ford succeeded to the deanship but because of his advanced age the duties of that office were practically turned over to Doctor Vaughan who became officially dean in 1891. His

services during the transitional period of the school form the subject of this paper, for it was during that time that his great influence came into being.

It is indeed remarkable that practically without any other training than that which he had acquired at Michigan, he should have recognized the full import of the scientific development which was then taking place and of the need of full-time men in the medical sciences. To one who was steeped in chemical laboratory methods, it was perhaps but natural to wish that the newer sciences should find their places in the medical curriculum.

Through his work in physiological chemistry he was in touch with the progress in physiology, which was then looming strong on the horizon as a result of the work of such men as Claude Bernard, Ludwig, and Foster. And, when in 1881 the question arose of an independent chair in that subject, young as he was, he strongly urged and secured the appointment of Henry Sewall, who proved to be an inspiring teacher and an investigator of the first rank. While with the University his work on immunization against snake venom opened the path that led to the production of immunity against the soluble bacterial toxins. Upon the resignation of Doctor Sewall, because of ill health, a successor had to be found, and Doctor Vaughan was fortunate in securing Doctor W. H. Howell, who, however, was soon called to Harvard and then to the newly organized Johns Hopkins Medical School. He in turn was followed, in 1892, by Doctor W. P. Lombard, who remained on the faculty until after the retirement of Doctor Vaughan.

When Dr. Howell came to the University, he was nominally given charge of histology, but the real conduct of that department fell to Doctor Huber, who has ever since maintained the high tradition of thorough laboratory work and productive research.

In the eighties the time-honored didactic teaching of *materia medica* was giving way to the new science of pharmacology. Doctor Vaughan's search for a thoroughly trained laboratory man resulted, in 1890, in the selection of John J. Abel as the first professor of pharmacology. Dr. Abel was a graduate of the University and had had years of training abroad and was therefore preeminently fitted for the new task. His fitness was such that upon the organization of the Johns Hopkins Medical School he was called and ever since has served there, enriching medicine by his masterful studies and deservedly acquiring the honor of being the foremost leader in his field. In 1893, his place was filled by the late A. R. Cushny, who, like Abel, was a pupil of Schmiedeberg. Cushny remained here until 1905 when he was called to London University and later to Edinburgh. Since then the chair of pharmacology has been admirably filled by his pupil, Dr. Edmunds, whose contributions have added prestige to the department.

In 1889, Doctor Vaughan faced the necessity of finding four men to fill the recently vacated chairs. One of these vacancies was due to the retirement of Dr. Sewall, and the appointment of his successor, Dr. Howell, has already been mentioned. Another chair to be filled was that of general chemistry and the happy choice of Dr. Vaughan in selecting Dr. Paul C. Frier, a pupil of the renowned Adolf Bayer of Munich, gave to Michigan a man who

by his training and skill contributed to a marked extent to the development of chemistry in the University. His enduring monument was the establishment of the Bureau of Sciences in Manila whither he was called in 1901.

In a similar happy manner came the selection of Dr de Nancrede as professor of surgery, and of Dr Dock as professor of internal medicine. These men were in every sense full time professors, for they devoted themselves unselfishly and whole heartedly to the clinical work of the hospital. They were an inspiration to the students and were held in the deepest respect by their colleagues. Doctor Dock was called to Tulane University in 1908, and his place was ably filled by Dr A W Hewlett until 1916 when he was called to Leland Stanford University. Dr de Nancrede ably assisted by his pupils, Darling and Lorce continued in service until his retirement.

When the chair of anatomy became vacant in 1894 by the death of that great and beloved teacher Corydon L Ford it was filled contrary to all previous practice, by a trained scientist, Dr J P McMurrich, who demonstrated that anatomy could be taught by a biologist irrespective of the possession of a medical degree. His great ability as a teacher and scientist was such that before long he was called to the chair of anatomy at Toronto University. He was followed in 1907 by Dr George L Streeter who in turn was called away in 1914 to the Carnegie Institute.

When the chair in pathology was vacated in 1895, Dr Dock became the nominal head, but the actual work devolved upon Dr Warthin who since then has developed a department second to none.

Several years before Dr Dock left the University, his pupil, Dr Cowie had already taken charge of pediatrics and in this field, by his ability and productiveness he has achieved signal success.

On the resignation of Dr Martin, in 1901 his place was filled by our honored colleague Dr Reuben Peterson. Similarly the withdrawal of Dr Carrow, in 1904, was followed by the appointment of Drs Canfield and Parker who have earned well deserved recognition.

The psychopathic hospital owes its existence to the wisdom and efforts of Dr Herdman. At his death in 1906, he was succeeded by Dr Barrett who, by his devotion to the work has made his department a model for others to follow. Subsequently the chair was divided and the chair of neurology was given to Dr Camp.

The vacancy resulting by the resignation of Dr Breakey in 1912 was filled by Dr Wile who by his contributions in his specialty has won the admiration of the profession.

I realize that the foregoing is but an inadequate expression of the efforts made by Dean Vaughan to gather a strong faculty. With unalloyed pride he often alluded to his share in this work.

The selection of the men I have mentioned during the crucial period of the eighties and nineties and in the years immediately following gave the medical school a commanding rank. There were however other conditions which had to be met. The three year course which had been in effect since

1880 was no longer adequate. In 1890 it was extended to four years in order to provide the fullest measure of scientific training with special emphasis placed upon laboratory instruction in the medical sciences. The laboratory method was extended to the clinical subjects by the organization of demonstration courses which aimed to impart to the student in the clinical years the same type of training as that which he had received in the fundamental sciences.

Dr. Vaughan realized that another step had to be taken to improve medical education and that was to increase the entrance requirements not for the purpose of reducing the number of students but rather to prepare them better for the work in medicine. The efforts of the best faculty would be largely mispent if the students were not adequately prepared. In 1890 a diploma from an approved high school was sufficient to meet the entrance requirements but in 1892, in addition, certain prescribed subjects, such as algebra, geometry, chemistry, physics, botany and zoology, were demanded and to these somewhat later trigonometry and a modern language were added. This progressive increase in the requirements finally culminated in 1909 in two years or sixty hours of collegiate work, including prescribed work in the languages and sciences. The number of hours required for entrance was eventually increased to seventy hours, or two and a half years of collegiate work.

Another significant step due to the initiative of Dr. Vaughan was the early establishment of the Combined Course. By arrangement with the Literary Faculty in 1892 students were permitted to register in the Medical School at the close of the third year and were given their bachelor's degree upon the completion of the first year in medicine. Eventually this arrangement was modified so that a student desirous of obtaining the two degrees could shorten the time from eight years to seven years for the A. B. degree and to six and a half years for the B. S. degree. By this arrangement the Medical School sacrificed nothing since the Literary College merely gave credit to which any student would be entitled if he elected such courses. The combined course eventually was adopted by many other universities.

The graduate work in the Medical School was encouraged in every way by Dr. Vaughan. As soon as he became dean, he obtained permission from the Board of Regents to enroll physicians and others in the various laboratory courses on the payment of a small fee.

Every effort was made by Dean Vaughan to provide his faculty with the best possible facilities for their work and in this he was highly successful. Funds were not plentiful but they were wisely used, and the results accomplished in these early years bore eloquent testimony to his foresight in the selection of his faculty.

Dr. Vaughan's service did not end with providing laboratory and clinical facilities. He knew from his personal experience that a good working library was a necessary part of the Medical School and very early in his career took an active part in creating the splendid Medical Library which the University possesses. Knowing as he did that the original sources were of the first importance, he saw to it that the library acquired complete sets of scientific

periodicals rather than a collection of textbooks. What he did in this direction was to him always a source of great pride.

The need of new hospital facilities was long evident. The old wooden pavilion hospital on the campus was to him a disgrace which had to be remedied. With that in view he appeared before the Legislature in 1889 and secured an appropriation of one hundred thousand dollars with which a new hospital was built. This was repeatedly enlarged, but by 1915 it was evident that a modern building was needed. The Legislature was again responsive and though the funds at first granted were inadequate the construction work on the splendid new hospital was well under way at the time of his retirement.

The medical laboratories in the nineties were largely in the old medical building which was erected in 1850. They were cramped and utterly inadequate. To remedy this condition Dr. Vaughan secured from the Board of Regents a modest appropriation with which the present West Medical Building was erected in 1903.

Dr. Vaughan's investigations began in the old chemical laboratory. By 1887 it was apparent that the quarters were inadequate for pursuing the problems pertaining to health and disease which were claiming his attention. His broad vision indicated the need of a separate institution. Accordingly, with the cooperation of the State Board of Health, the Legislature of 1887 was memorialized to establish a State Hygienic Laboratory at the University. The object of this laboratory, as stated at the time, was first, to study the causation of disease; second, to make analyses of food and drinking water; and third, to teach the causes of disease. The request was granted and an appropriation of forty thousand dollars was made for the erection of a building to be used jointly by the Department of Physics and the Hygienic Laboratory. At this time some attempts were made in the old laboratory to apply the new science of bacteriology to the solution of problems arising in connection with the examination of waters, but it was seen that a thorough training in the new discipline was necessary. At that early period this could only be obtained in Germany. Accordingly, Dr. Vaughan spent the summer of 1888 in Koch's laboratory in Berlin where under the direction of Carl Fraenkel a first hand knowledge of the new methods was acquired.

The Hygienic Laboratory at the University was completed in the fall of that year, and it was opened for work in January, 1889. It was the first laboratory in this country which offered systematic teaching of bacteriology to physicians and students. Before long the laboratory outgrew its quarters and in 1903 it was moved to the new, the present West Medical Building and since 1926 it occupies a wing in the East Medical Building. For twenty years after the opening of the laboratory, Dr. Vaughan was active as its director and it was during this period that a further and important step in extending its service to the state took place. In 1903, on the occasion of the first serious outbreak of rabies in the state, Dr. Vaughan obtained from the Board of Regents authorization to establish a Pasteur Institute as a part of the Hygienic Laboratory. At that time the antirabic treatment was not given except in two or three places in this country. Undoubtedly many lives have been saved through his wise foresight.

Apart from the Medical School Dr. Vaughan exerted a lasting influence on medical education in the country at large. He was an active participant in medical meetings and gave generously of his time and energy to promote medical education. His services were constantly in demand. He was a member of the Council on Medical Education from 1904 to 1913, and was Chairman of the Council on Health and Public Instruction from 1919 to 1923 and the last year in this capacity he devoted as editor to the newly-established journal, *Hygeia*. For several years he was Chairman of the Medical Division of the National Research Council.

I must forego even an enumeration of his many activities in promoting medical education at large. He was recognized as a great leader, a constructive thinker, and a broad idealist. By his students he was beloved and respected and to his colleagues who knew him best he was a man—honest, upright, and sincere, whose every effort had as its objective the good of the University which he loved as long as he lived.

—*Fredrick G. Novy,*
Ann Arbor

DOCTOR VAUGHAN'S WORK IN MEDICAL CHEMISTRY*

DOCTOR VAUGHAN came to Ann Arbor in 1874 entering at once upon the study of chemistry. His student days fell in that period when the opinion still prevailed that the medical department in Ann Arbor consisted of a chemical laboratory with a medical school attached to it. Our early chemistry teachers—Douglas, Prescott, Rose, Langley—were all graduates in medicine. Naturally, the practical phase of chemistry was receiving at that time by far the major attention in our laboratory—the analysis of plants and foods, of drugs and poisons.

Presumably because of this intimate connection between the Medical Department and chemistry Vaughan the young assistant in the chemical laboratory after completing in 1876 the work for the Ph.D. degree took up the study of medicine. Two years later he graduated from that department. For the next six years his duties were divided between the Chemistry Department and the Medical School but even after his appointment 1883 to a full professorship in the Medical School his working quarters remained in the Chemistry Building. In fact it was not until 1889 that Dr. Vaughan moved into the newly equipped Laboratory of Hygiene and Bacteriology which occupied the third floor of what is at present known on the campus as the West Physics Building.

Thus fifteen years of his early active life—1874 to 1889—were spent in the old chemical laboratory with chemists as associates. It was in this building in a small, crowded room on the second floor in a room provided with only one window that his first fundamental researches were done on the separation and identification of inorganic and organic poisons. These researches attracted wide attention and placed him at once among the leading authorities in toxicology, a position which he retained to the end of his life. Here in this cramped space were initiated the studies on the contamination of drinking waters and the results of these studies proved of inestimable value to many communities throughout the State of Michigan. Here also was commenced the important investigation that attracted so much attention in its day, namely the investigation concerning the occurrence and the chemical composition of the poisonous constituent that is formed in cheese, milk, and cream, and to which Dr. Vaughan gave the name "tyrotoxin."

The beginnings of his extensive literary labors were also started while he was still in the old chemical laboratory. In addition to a large number of smaller publications in medical journals he published texts on physiology, pathology, and materia medica and in each instance the chemical viewpoint was emphasized. One text was entitled *Chemical Physiology and Pathology* and went through three editions in two years 1878-1880. The treatise entitled

Potomains and Leucomains or the Chemical Factors in the Causation of Disease (by Vaughan and Novy) went through four editions.

Courtesy of Wilfred B. Shaw, Director of Alumni Relations, University of Michigan

After removal to the new Hygiene Laboratory, in 1889, bacteriology, then a comparatively new science, engrossed his major interests. Here for fourteen years, and for almost twenty years longer in the West Medical Building, were accomplished a series of investigations that stamped Dr. Vaughan's laboratory as the most productive place on our campus. A large number of graduate students were attracted, and the influence that emanated from that laboratory proved of the greatest value for the growth of graduate work in every other department of the University. It became definitely understood on our campus that in the Medical Department the policy was well defined: teaching and research must go hand in hand.

But while, in the new Hygiene Laboratories, bacteriologic problems were the main focus of attention, the chemical aspect was never left out, and this imparted to Dr. Vaughan's investigations a feature which differentiated them in a striking manner from work along similar lines in other institutions. In his studies on the germicidal action of serum, in the investigations of the bacterial poisons and of the poisons from natural proteins in questions of immunity and of sensitization—in the pursuit of all these problems he was both bacteriologist and chemist.

His work became highly regarded by the chemical profession, not only because of the intrinsic value of the specific contributions themselves, but also because these contributions emphasized strikingly the important services of chemistry in the solution of medical problems. Indeed, as far back as 1891, he defined his views in this respect in a paper published in the medical journals and entitled "The Growing Importance of Chemical Studies in Medical Education and in Medical Research." By precept and by example he preached this doctrine throughout the succeeding thirty years of his deanship.

In common with other students of biology, gifted with active mind and vivid imagination, how could Dr. Vaughan have evaded the insistent question—what is the origin of life? Having to deal daily as he did with the lowest forms of things living, so small as to be filterable through porcelain, so low in the scale of life as to be nonvisible even through the most powerful microscope, it was not possible to avoid the query—where is the line of demarcation between inanimate and animate matter? What is the mysterious force that causes the conversion of the former into the latter? This inscrutable problem had an irresistible fascination for him. And when, two years ago, the American Chemical Society invited Dr. Vaughan to deliver the principal address at the Society's annual meeting, the address bore the significant title "A Chemical Concept of the Origin and Development of Life." One is not called upon necessarily to agree with the hypothesis therein developed so plausibly and even brilliantly. No one, however, can read that paper without becoming deeply impressed by the writer's extraordinarily wide knowledge of the most recent developments in every branch of science, and by his keen powers of analysis and synthesis.

For one who has had the good fortune to know Dr. Vaughan personally, it is impossible to speak of him in the merely abstract manner, to speak of his having been a very eminent man in the science of medicine, in medical educa-

tion, in chemistry. He was all that—and very much more. His warm and impulsive, always generous, personality has been a most potent factor in the life of the University. He had a sympathetic interest for, and quite an intimate knowledge of, scholarly and creative work by the members of all the various faculties of our University. He gave friendly and inspiring encouragement to many and many a struggling beginner. He rejoiced in the achievements of others. We have all known him as a truly great man and also as a generous and good man. We shall revere his memory.

—Moses Gomberg
Ann Arbor, Mich



COLONEL VAUGHAN 1919

VICTOR CLARENCE VAUGHAN AS A BIOCHEMIST

ALTHOUGH the enviable reputation of Dr Victor C. Vaughan is dependent largely upon his scientific contribution to bacteriology and public health and to the fact that as dean he developed one of the great medical schools of this country in a small middle western town his first interest in science came to him through chemistry and throughout his life he held the point of view of a chemist in the many problems he undertook. As is so often the case, his interest was turned to chemistry quite accidentally.

After graduation from Mt. Pleasant College in 1872 he was appointed professor of Latin. In an unused room in the college, which had been closed during the Civil War, Victor Vaughan discovered a number of unopened packing cases which on investigation proved to contain a complete outfit for a chemical laboratory. Obtaining permission to set up a laboratory, and to experiment with the various chemicals he soon became fascinated with the work and within a short time was teaching chemistry along with his Latin.

In 1874, he entered the University of Michigan to pursue graduate work in chemistry, receiving the M.S. degree in 1875 and the Ph.D. degree in 1876. After two years' further study he received the degree of M.D. As early as 1875 Dr. Vaughan began to take part in the teaching in the medical school as instructor in medical chemistry. He was made lecturer in 1879 and assistant professor in 1880. In 1883, he was promoted to a full professorship with the title of professor of physiological and pathological chemistry. For fifteen years (1874-1889) Dr. Vaughan carried out his researches in the chemical laboratory. In 1887 after the construction of the Hygiene Laboratory was authorized his title was changed to that of professor of hygiene and physiological chemistry and director of the hygiene laboratory, a title which he retained until his retirement in 1921.

Dr. Vaughan's early work in biochemistry was done contemporaneously with that of Atwater at Wesleyan and Chittenden at Yale and he was thus one of the first workers in this new field in this country. He was undoubtedly the first to hold a chair of physiological chemistry in a medical school in this country and to give chemical instruction from this more modern point of view.

Although the *Journal of Biological Chemistry* and the American Society of Biological Chemists were not founded until after Dr. Vaughan's interests had been turned quite largely toward bacteriology, still he was active in both of these undertakings. He was a collaborator of the *Journal of Biological Chemistry* from its founding in 1905 until 1920 and a charter member of the American Society of Biological Chemists in 1906 and one of its officers in 1910. The early volumes of the *Journal of Biological Chemistry* contain a number of important contributions by Dr. Vaughan's pupils, in particular upon the chemistry of bacteria and bacterial proteins.

In 1878 he published a textbook on "Chemical Physiology and Pathology" which went through three editions in as many years. Later he issued a quite comprehensive text on "Physiological Chemistry" in mimeographed form in order that he might have a book readily adaptable to the needs of his own students. He was early attracted to the field of nutrition and published "Balanced Diets" in 1887. Twenty-five years ago every student of bacteriology was familiar with Vaughan and Novy's "Cellular Toxins" which went through four editions. This book possessed a chemical background which could only have been given to it by men of thorough chemical training. In 1916, Dr. Vaughan was invited to give the Heiter lectures at the University and Bellevue Medical College. These were subsequently published in book form under the title of "Poisonous Proteins." The writer well remembers the inspiration he received from these lectures, which beautifully summarize some of Dr. Vaughan's most important biochemical researches.

Despite his many administrative duties Dr. Vaughan not only found time to conduct research but by so doing furnished a very great stimulus to his students and colleagues. Of his numerous publications in scientific journals the larger number clearly show the influence of his chemical training. In connection with his work in bacteriology it is interesting to note that like Pasteur he was recruited from the ranks of the trained chemists. He apparently not only held the first chair of physiological chemistry in a medical school in this country, but organized the first bacteriological laboratory.

As one goes over some of his bacteriological publications in the early nineties, his chemical line of thought is evident. Such titles as the following may be mentioned: "Some new bacterial poisons," "The germicidal properties of nucleins," "The principles of immunity and cure in infectious diseases," "The nature of the germicidal constituent of blood serum." His work on tyrotoxin, a poisonous substance which he found elaborated in cheese is well known, as is his work on ptomaines, toxins and leucomaines. Later the energies of Dr. Vaughan and his coworkers were devoted very largely to a study of the chemistry of the bacterial cell, and this work and related studies, as brought out in his Heiter lectures on poisonous proteins, was probably his most important biochemical contribution. He considered the influence of these various factors in relation to fever, the phenomena of anaphylaxis, immunity and disease.

The inspiration which he received from his early chemical training followed him even after his retirement, as is evident from the titles of two lectures which he gave in 1927. These are "The chemistry of living substance and its adaptability to its environment," the third Kober lecture given March 28, 1927, and "A chemical concept of the origin and development of life," an address at the seventy-third meeting of the American Chemical Society, April 13, 1927. One cannot read these papers without becoming deeply impressed by Dr. Vaughan's extraordinary, wide knowledge of the most recent developments in every branch of science.

—Victor C. Myers,
Cleveland

VICTOR CLARENCE VAUGHAN AS A TOXICOLOGIST AND MEDICO LEGAL EXPERT

WITH or without our consent the master whom we meet leaves a lasting impression upon our souls. And long is the list and sincere is the respect of those who must thus acknowledge their indebtedness to Dr. Vaughan. Born to the trials and tribulations of a warring, backwoods Missouri district, steeped in Latin and trained in chemistry, Dr. Vaughan emerged like a rising star out of a darkness and an obscurity from which only the hand of Providence (to quote his Huguenot ancestors) could have rescued him from eternal oblivion. And none knew this better than Dr. Vaughan himself who sagely remarks that by a slight turn in the circumstances of his early environment he might easily have become a western cowboy. Such is the stuff from which come those who erect the signboards of civilization.

With this magnificent background a B.S. degree from Mt. Pleasant College (Mo.), an M.S., a Ph.D., and an M.D. from the University of Michigan gave Dr. Vaughan a scientific training of the first rank. All of these degrees were granted to him between the years 1872 and 1878. But the real foundation of Dr. Vaughan's future greatness as an epidemiologist, a toxicologist and a medico-legal expert lay in his early and extensive experience as a teacher, first of Latin, then of chemistry (eight years), medical chemistry (one year), physiology (three years), physiological and pathological chemistry and materia medica (four years), and finally as director of the hygienic laboratory and professor of hygiene and physiological chemistry for twenty-two years. A vigorous and conscientious student, Dr. Vaughan developed rapidly and with great promise from the very start of his teaching career. Only those who in the classroom have faced large numbers of keen and alert medical students can fully appreciate the stimulus which this experience afforded Dr. Vaughan to work out and completely master every detail of the chemical, toxicological, physiological and pathological problems which he attempted to present to his classes. His knowledge of toxicology was enormously enhanced by great numbers of experiments which he performed, either for or with his students each year. These experiments gave him a fund of first-hand experience which has probably never been surpassed by any individual worker. For his classes covered not only the chemical but the toxicological and pathological phases of the problems as well. Cautious and always extremely critical of his own work, widely conversant with the literature and stimulated by his close association with many able colleagues and eager students, Dr. Vaughan used these experiences as the foundation upon which he rose to the highest pinnacle of eminence as a toxicologist and medico-legal expert.

While still a very young man his reputation as an expert along these lines began to reach out to more and more distant points, and the demand for his services became more and more insistent. The part played by bac

teria in many epidemics, in food poisoning, and in the pathology of various obscure individual cases was not fully appreciated in the earlier years of Dr Vaughan's work. This frequently led to confusion between cases of true chemical poisoning and those due to bacteria. In fact in the earlier years of Dr Vaughan's scientific career the "germ theory" of disease was by no means universally accepted. And much of the work, and many of the original investigations, carried out in Dr Vaughan's own laboratory, were devoted to clearing up the relations existing between true chemical poisoning and that due to living organisms, especially in epidemic form. Among Dr Vaughan's earliest papers were articles dealing with the separation of arsenic and antimony. And much of his research dealt with other mineral, vegetable, bacterial and animal poisons. For the study of poisons, their chemistry, and their effects on living things, always held a peculiar fascination for him. And his work as a toxicologist and medico-legal expert nearly always dealt with this phase of the subject. A vigorous, scientific contest in one of these legal cases gave him a striking thrill and inspiration which he usually enjoyed immensely.

His long services as a member of the Michigan State Board of Health gave him many opportunities to benefit the public in the way of introducing, or of directing attention to, needed reforms in the preparation or preservation of food supplies, the purification of water supplies, or in the use and distribution of various poisons, such as the employment of sulphite in preserving meat, or of other chemicals for preserving milk.

Dr Vaughan took part in a great number of medico-legal cases and among those which he himself considered most important may be mentioned the Hall case and the Millard case, both of these dealing with the postmortem imbibition of arsenic. Experiments performed by Dr Vaughan in the latter case demonstrated conclusively (as had earlier been shown by Orfila and by Kidd) that arsenic will diffuse from a localized point throughout all the tissues of a dead and buried body. These cases had much to do with the enactment of laws prohibiting the use of arsenic in embalming fluids and on the exercise of greater care on the part of druggists in dispensing poisons. The Carveth case, the Hughes case, the Buchanan case, the Fleming case and the Waite case were others which stood out particularly in Dr Vaughan's own memory. But it is probable that the coco-cola case, the benzoate of soda dispute, and the legal status of saccharine made the greatest impressions of all on the general public.

Dr Vaughan learned much of the tricks, slips and dishonesty of the representatives, or misrepresentatives, of the law in these extensive experiences. And he not infrequently refused to serve in a case concerning the justice of which he was not certain, or in which his evidence might go against the deserving side. And on many occasions he took great pleasure in voluntarily giving the benefit of his experience and advice where these had not been sought in order that those who deserved help might receive it. Mentally he usually felt at his best on the witness stand, and among those who appeared, either with or against him, in numerous legal contests might be mentioned a long list of the most brilliant and renowned medico-legal experts of this coun-

try and some from Europe. Among these were Haines, Witthaus, Doremus, Hektoen, Prescott, Peterson, Le Count Underhill, Benedict, Schultze Valentine Mott Jr. Wolff, Liebreich, Wiley, Langley, Kedzie and many others. His long and varied experience as an expert witness led him to formulate certain rules for his own guidance in these matters. These were, first never to accept service in a case unless he was convinced that there was scientific justification for the claims on his side, second to avoid all sentiment in presenting his testimony, third to be extremely modest in giving his qualifications as an expert, fourth to maintain his good humor and not to assume a resentful or hostile attitude toward opposing counsel or on cross examination, fifth to insist on his right to qualify his answer if he preferred to make it more extensive than "yes" or "no" and sixth to express no opinion as to the guilt or innocence of the person on trial.

Dr. Vaughan's work covered an enormous range and variety of subjects. In addition to more scientific definitions he used to tell his students that hygiene covered any subject about which he wished to talk. But throughout all of his investigations he never lost sight of the chemical and the toxicologic aspects of the problems involved.

While the list of his publications which dealt either directly or indirectly, with toxicology and forensic medicine is very long I need mention but a few here. Aside from his books on *Physiological Chemistry* (1878-80) *Cellular Toxins* (Vaughan and Novy, 1902) *Protein Split Products* (1913) *Epidemiology and Public Health* (1923) Dr. Vaughan contributed extensive special sections on the general field of toxicology to the *System of Legal Medicine* by Allan McLane Hamilton and Lawrence Goodwin (1894) to the *American Textbook of Pathology* (1902) to *Forsheimer's Therapeutics of Internal Diseases* (1915) and to the *Legal Medicine and Toxicology* (1923) by Peterson Haines and Webster. As Dr. Hektoen has noted Dr. Vaughan served as a connecting link between the period of the sanitary chemist and that of the modern bacteriologist and he was familiar with the methods of both.

In the year 1885 Dr. Vaughan arrived at the conclusion that he had found in poisonous cheese a new chemical compound resultant on bacterial action to which he gave the name "tyrotoxinon." In carrying on these investigations Dr. Vaughan in common with all other scientific workers of the time was compelled to labor in much darkness with reference to the great diversity and toxicological possibilities of the bacterial inhabitants of the cheese or other milk products in which he concluded tyrotoxinon might exist. Strenuously and persistently and with the zeal of the true scientist he strove to isolate this poison in pure form. It is probable that no single ambition clung more tenaciously to his subconscious hopes than that of his desire finally to isolate and study this compound. For perhaps nearly twenty years his thoughts from time to time reverted to the isolation of tyrotoxinon. At one time he believed it was diazobenzene hydrate ($C_6H_5N_2OH$), but later the evidence seemed to indicate it might be diazobenzene butyrate. With the passing of time and with the vast advance made in bacteriological knowledge it now seems probable that such a pure, simple compound as tyrotoxinon, as Dr. Vaughan conceived it does not exist,

teria in many epidemics, in food poisoning, and in the pathology of various obscure individual cases was not fully appreciated in the earlier years of Dr Vaughan's work. This frequently led to confusion between cases of true chemical poisoning and those due to bacteria. In fact in the earlier years of Dr Vaughan's scientific career the "germ theory" of disease was by no means universally accepted. And much of the work, and many of the original investigations, carried out in Dr Vaughan's own laboratory, were devoted to clearing up the relations existing between true chemical poisoning and that due to living organisms, especially in epidemic form. Among Dr Vaughan's earliest papers were articles dealing with the separation of arsenic and antimony. And much of his research dealt with other mineral, vegetable, bacterial and animal poisons. For the study of poisons, their chemistry, and their effects on living things, always held a peculiar fascination for him. And his work as a toxicologist and medico legal expert nearly always dealt with this phase of the subject. A vigorous, scientific contest in one of these legal cases gave him a striking thrill and inspiration which he usually enjoyed immensely.

His long services as a member of the Michigan State Board of Health gave him many opportunities to benefit the public in the way of introducing, or of directing attention to, needed reforms in the preparation or preservation of food supplies, the purification of water supplies, or in the use and distribution of various poisons, such as the employment of sulphite in preserving meat, or of other chemicals for preserving milk.

Dr Vaughan took part in a great number of medico legal cases and among those which he himself considered most important may be mentioned the Hall case and the Millard case, both of these dealing with the postmortem imbibition of arsenic. Experiments performed by Dr Vaughan in the latter case demonstrated conclusively (as had earlier been shown by Orfila and by Kidd) that arsenic will diffuse from a localized point throughout all the tissues of a dead and buried body. These cases had much to do with the enactment of laws prohibiting the use of arsenic in embalming fluids and on the exercise of greater care on the part of druggists in dispensing poisons. The Carveth case, the Hughes case, the Buchanan case, the Fleming case and the Waite case were others which stood out particularly in Dr Vaughan's own memory. But it is probable that the coco-cola case, the benzoate of soda dispute, and the legal status of saccharine made the greatest impressions of all on the general public.

Dr Vaughan learned much of the tricks, slips and dishonesty of the representatives, or misrepresentatives, of the law in these extensive experiences. And he not infrequently refused to serve in a case concerning the justice of which he was not certain, or in which his evidence might go against the deserving side. And on many occasions he took great pleasure in voluntarily giving the benefit of his experience and advice where these had not been sought in order that those who deserved help might receive it. Mentally he usually felt at his best on the witness stand, and among those who appeared, either with or against him, in numerous legal contests might be mentioned a long list of the most brilliant and renowned medico-legal experts of this coun-

VICTOR CLARENCE VAUGHAN AND HIS WORK AGAINST TUBERCULOSIS

IT IS commonly believed that the net effect of a person's accomplishments on human society can only be evaluated some considerable time after his personal influence has ceased to be felt. Lives recorded on this basis allow great economy in historical storage, since beyond a decade or two of a man's exitus what remains of his specific work rarely requires more than a statistical line. Such records have a palaeontologic value but they contain no hint of the play of living forces that made them possible. What common people call the soul of man is equipped with hereditary attributes which determine his individual responses to environment. Among these intangible assets are potent *aspiration, ambition, will, courage, tenacity, affection, character*. These among others are the forces that determine the human career, which make up the real man, which can instruct, guide and warn the student of biography. From this point of view the net accomplishments of a life are less important than a knowledge of how they were obtained.

The charming account of his own history¹ which Vaughan has left us has only implied the courage, tenacity and consistent purposiveness with which his designs were pursued. But its lack of egoism gives no direct hint of the aggressive power of the man, of his vision of the trend of events, of his keen judgment of men and his ability to secure their cooperation or override their opposition in the policies which his mature judgment dictated. Through devotion to science a great politician perhaps a great statesman was in him lost to the country.

The autobiography represents a youth between the ages of sixteen and twenty three studying and teaching in a series of junior colleges in Missouri. Following on the heels of the Civil War this seven years of education knew nothing of modern pedagogic facilities or technique. Few of the teachers themselves were expertly versed in their subjects but the spirit was there, the lust for understanding in the pupil.

Probably every man who has emerged from the crowd has been able to point out in his long list of instructors one or a few to whom his intellectual and moral debt was exceptionally great. Such a teacher Vaughan encountered when in his seventeenth year he came under the domination of the Rev. J. W. Terrill. Quoting Vaughan: "The college was a one man institution and James W. Terrill was the greatest educator I have ever known but like many others he was only great in the face of obstacles and became weak when these were removed." The theory of President Terrill was that no one knows anything until he can clearly state it in writing. His criticisms of the

¹ A Doctor's Memories. Bobbs Merrill. Indianapolis. 1906

selection of words was scathing. He was wont to question even the most direct and correct statements. In doing this he felt at liberty to resort to the worst kind of sophistry. Nothing delighted him more than to make a good student acknowledge his error when in fact he was right. Then with great glee he would point out the fallacies in his own argument and chide the student for being so easily browbeaten. He taught by disputation, a method of education beloved by the ancients but now fallen into desuetude. It has been of service to me, especially when on the witness stand. The only direct instruction I had from President Terrill was in Latin and what was then called 'mental and moral' philosophy. There were ten in the class equally divided between the sexes. Often the hour closed after the statement of eleven different religious creeds. The one good effect this course had on me is that I had never since combated anyone's religious belief. President Terrill made no pretension to a thorough knowledge of Latin and he plainly told me of his limitations the first day I met him. In fact his reading in this language had scarcely exceeded mine at the time. With this knowledge I accepted him as a teacher and I admit that for the first few months I received from him the most atrocious flagellations that were ever showered upon my shoulders by a teacher. Up to that time I had known only the so-called English, more correctly Scotch, pronunciation. At this he hurled all forms of ridicule. At last after weeks of trial the devil was exorcised and I was congratulated on being fully prepared to converse with Cicero without giving him torture after old Chaon had ferried me across the Styx."

When he entered Mt Pleasant College, Vaughan had but a vague idea of physics and chemistry though at his home there were some old illustrated books on natural history which he had read with eager wonder.

One door in the college had always been locked until Vaughan obtained permission to investigate. Behind it he found a small room with shelves on which were numerous labeled bottles of pure chemicals. It was a miniature chemical laboratory of prewar days. "With Barker's *Chemistry* and its clear statement on nomenclature, it was easy to ascertain the composition of the contents of the bottles and to perform simple reactions such as the precipitation of soluble salts of silver and lead with sodium chloride. The first time I made hydrogen sulphide the odor penetrated the whole building, and my embryonic chemical studies were threatened with complete annihilation. However I learned discretion and finally I had permission to offer a course in elementary chemistry, at first limited to two or three students. During my last years at Mt Pleasant I came into possession of a copy of Douglas and Prescott's *Qualitative Analysis* and thus decided the question long debated in my mind as to whether I should choose classics or science for my life work, and where my education should be continued when I left Mount Pleasant. After my graduation (in 1872) I continued teaching Latin and Chemistry until February, 1874. Then came the inevitable break with President Terrill."

The foregoing outline includes the first stage of Vaughan's education. Its definite acquisitions consisted in a fair acquaintance with Latin and a limited knowledge of practical chemistry. The former must probably be

given credit for that forceful and graceful diction which later marked his spoken and written words, the latter was the solid corner stone of his future career

But already were manifested the traits of the coming man. A personality aggressive and courageous to a degree, an alert intellectual curiosity, a keen responsiveness to the stimulus of difficulty and opposition, insistence on independence of judgment and individual mastery of understanding. But already, too, there was evidence of that yearning which seems to be exceptional among scientific men, especially those of laboratory training, namely the humanistic impulse to know, to influence and to consort with his fellow men.

At the age of twenty three, in the fall of 1874, Vaughan sought to pursue his education at the University of Michigan.

Like the rest of his countrymen with similar designs, he realized that the first great educational milestone to pass was an academic degree. He desired to concentrate on chemistry as a major, with geology and biology as minor subjects. Even in those days the irregularity of his preparation disqualified him for admission to the graduate school. But President Angell, with that vision for which he was famous, unhampered by convention allowed the decision as to qualifications to be referred to a committee of three of the Faculty. The candidate, evidently sensing the proclivities of his examiners, realized that his ominous weakness lay in the field of crystallography, a specialty with one of them. His mode of reaction was characteristic. He obtained a half bushel of potatoes and with his knife reproduced from them all the crystal forms described in Dana's *Mineralogy*. The outcome was the acquisition of the degree M.S. in 1875, and of Ph.D. in 1876 and his entrance as a student in the Medical School in the fall of the latter year.

All this time Vaughan was student and assistant in the chemical laboratory. Those who knew both men may suspect that the gentle, lovable and "square" chief of the department of chemistry, Professor Albert B. Prescott, must have exercised a very salutary influence over his younger colleague.

In the year preceding his matriculation as a medical student there was an upheaval in the chemical staff leading to dismissal of one of the principal teachers. Vaughan was appointed to fill the vacancy as Instructor in Physiological Chemistry. This office involved the soul trying experience of lecturing to a horde of medical students who had their own views on the propriety of the new appointment, who were predisposed to turn "thumbs down" on the incumbent, in which case his future must have been passed in an advanced stage of purgatory. But through infinite tact and unsparing preparation Vaughan at once won popular indorsement from that critical and hard fisted group of which he was soon to become a junior member. In 1878 he put in book form his lecture notes on physiological chemistry and of this there were published three editions in as many years.

During this time he must have been admitted as a junior member to the meetings of the medical faculty, the ruling forces of which were on the purely clinical side of medicine and represented in Practice by A. B. Palmer, Dean,

in Surgery by Donald Maclean and in Ophthalmology by G E Frothingham, an outstanding group of dominating personalities

This was a period of fomenting unrest among the better minds of the medical profession over the inadequate standards of medical education. It was realized that a deficiency existed especially in the personnel and facilities necessary to the proper teaching of the Institutes of Medicine, whose foundation was recognized as of animal physiology. Henry Newell Martin, a product of the modern renaissance in English physiology, had been sent by Huxley and Foster to head the department of Biology in the newly founded Johns Hopkins University, in 1876. Martin soon established a laboratory course definitely designed as "preliminary to the study of medicine." This was nearly thirteen years before the opening of the Johns Hopkins Hospital and seventeen years before the beginning of its Medical School. The present writer had the good fortune to be Assistant in that laboratory.

Martin's extra-mural teaching was through example rather than precept and its influence upon the subsequent development of medical education in this country was beyond estimate.

Vaughan, though only of the rank of Assistant Professor, had acquired such respect in his Medical Faculty that he was given the initiative in plans to strengthen the "scientific" side of the medical curriculum. His first move was for the establishment of a special department of Physiology. In later years, Pathology, Anatomy, Pharmacology and Preventive Medicine were in turn impressed or created by his touch.

Principally through the efforts of V C Vaughan the present writer became the first incumbent of the chair of Physiology in the University of Michigan, in the Spring of 1881.

apparently first put him in touch with German literature and German men of science. The scientific Crusoe in the rural town of Ann Arbor reached out through the radio substitute of those days and came into communication, as later he did in personal contact, with the old world champions of science.

Of great value in the development of his tastes and in the focalizing of his knowledge must have been the self culture emanating from an elective course of lectures on Sanitary Science which was offered annually by Vaughan and to which all University students were eligible. These courses were initiated some time in the early '80's and became exceedingly popular. They played the part of scientific *conversations* in which Vaughan interpreted to laymen the fast surging world thought of medical biology through the code of his own experience. He was an impressive and convincing speaker. Sitters in the back row missed nothing of his discourse. In his own words, 'I counted a lecture hour wasted if I did not know more about the subject myself when I finished than when I began. As I proceeded in each lecture I saw my subject in a broader or at least in a modified form, or there flashed upon me some better way of presenting the facts or making them more comprehensible to my students.'¹ The important connotation is that for him scientific facts gained their interest from their bearing on human welfare. Hence his application of the tyrotoxicin discovery to general food poisonings and as a key to the summer diarrheas of children. It led him with his pupil colleague and later successor F. G. Novy, to put forward in 1888 a critical summary of existing knowledge on organic poisons, exogenous and endogenous (followed by two other expanding editions)², and later, assisted by his elder sons, led him to produce one of the most original and clearest of all monographs devoted to the infant science of immunology.³

Vaughan's constant productive activity in the field of chemical bacteriology as applied to public health was one of the most important educating influences in the eighth decade of the last century. A critical scientific estimate of his work was signalized by his election to the Association of American Physicians in 1889 succeeded in 1915 by the extraordinary distinction of Honorary Membership.

Vaughan seems to have practiced medicine from the date of his graduation. In this responsive field he revelled in that 'human touch' which vitalized his laboratory conceptions. Onlookers wondered at the energy that was distributed with adequate intensities between academic administration teaching in the lecture room and laboratory, a large private and consulting practice, literary composition and above all, original research. Yet he rarely seemed tired, and never in haste.

In that period, tuberculosis stood alone at the head of all mortality tables. In the Registration Area the death rate per 100 000 population was in 1900 201.9, in 1920 it had dropped to 114.2.

¹ Vaughan V. C. and Novy F. G. *Pyomaines, Leucomaines and Bacterial Proteins* vol. 1 1897.

² Vaughan V. C. V. C. Jr. J. W. *Protein Split Products* 1911.

Villemin, Pasteur, Koch had set up a shrine whose oracles spoke words of hope and understanding that thrilled every scientific student of disease

Vaughan but followed the natural law of his being when his interest and his energy became more and more definitely focused upon tuberculosis, especially in its pulmonary form, in both its social and its scientific aspects. He was like a radioactive element distributing the truth within him. His public addresses were powerful appeals for rational and practical tuberculosis control.⁴ It was educative influences like his that led to the founding in 1904 of what is now the National Tuberculosis Association, in which he was a member of the first Board of Directors and President in 1919.

But with his ideals, tastes and training it would have been strange had Vaughan been content to regard tuberculosis as merely a forensic subject. Koch had disclosed the tubercle bacillus in 1882 and then, in 1890, announced its conqueror in tuberculin. This misconception of the remedy still awaits solution.

The world of scientific medicine was at a loss. The attacking agent in tuberculosis was known, but the conditions of *vulnerance* in this and other microorganisms, on the one hand, or of *resistance* in the invaded host on the other, had in no way been formulated. It is true that Salmon and Smith, in 1886, as a conclusion from their researches on hog cholera had definitely stated that immunity may be produced by introducing into the animal body the chemical products of bacterial growth,⁵ and in 1887 Sewall had demonstrated that pigeons, following serial sub-lethal inoculations with rattlesnake venom, could be protected against 7 times the fatal dose after a resting period of at least 5 months,⁶ and that later, 1894, Calmette⁷ and others, using cobra venom, prepared from the blood of inoculated animals a protective and curative anti-venom. But there was little known of the data of immunity and there was no general theory of the subject.

Nevertheless the ten years following 1890 may be called the Elizabethan period of bacteriology and immunology. As with miners in a gold rush, a "let's go" spirit enthused an unexampled swarm of finest intellects from well-nigh every land encircling the globe, all intent on finding the meaning of life from its lowest forms, in seeking to understand and control infectious disease. No proper estimate of those activities can be made by one unacquainted with the spirit of the times. Scientific ideals evoked an international enthusiasm comparable to the patriotism roused by sight of the national flag.

It was a period of intensive truth-seeking production in a virgin field. Remarkable, indeed, is the permanence and basic importance of the discoveries made in those days. The tremendous tome of Straus, after thirty-five⁸ years, is delightful reading today and, for its period, as safe as now current literature.

⁴Cf. The Problem of Tuberculosis. Wis. Med. Jour. July 1905.

⁵A New Method of Producing Immunity from Contagious Disease. Proc. Biol. Soc. Wash. D. C. III 1884 6 p. 29 printed Feb. 22 1890. (Ref. from Zinsser in Infection and Resistance p. 72.)

⁶The Preventive Inoculation of Rattlesnake Venom. Jour. of Physiology 1887 VIII 203.

⁷Calmette A. Compt. rend. de la soc. de Biol. 1894.

⁸Straus I. La Tuberculose et son Bacille 1895.

It was in this period that V. C. Vaughan at early intellectual maturity, entered the arena against tuberculosis. Vaughan's studies had led him to vaguely anticipate the chemotherapeutic idea, the effective agent of his conception finding its origin in the body of the host.

By an elaborate course of reasoning checked by experimental data disclosed in current literature Vaughan adopted certain tenets to guide his investigations thus: 1. Active immunity must be cellular in origin. 2. "Physiologically, nucleins may be said to form the chief chemical constituents of the living parts of cells. Speaking broadly we may say that the nuclein is that constituent of the cell by virtue of which the histological unit grows, develops and reproduces itself. It is the function of the nuclein to utilize the pabulum within its reach. It must be evident that those tissues most abounding in cellular elements contain relatively the largest amount of nuclein. It must also be seen that it is by virtue of their nuclein that the cells of various organs and organisms possess and manifest their individual peculiarities." 3. "I am ready to believe that the immunizing substance (against bacterial disease) is a constituent of the bacterial cell itself. I believe it is the nuclein." 4. "the cause that brings into existence the condition of immunity is a bacterial proteid. Now in order that this exciting cause may induce the condition of immunity, it must act upon something. Upon what organ of the body does it act?" "The cells upon whose altered activity immunity depends are probably those of the spleen, the bone marrow, the thyroid and thymus glands and possibly other glandular organs. In what way are these organs concerned in the production of immunity? do they elaborate antitoxins and if so what can be said about the nature of these antitoxins?" I have borne in mind the fact that these organs are the sources of the nucleated white blood corpuscles. Do these corpuscles contain germicidal or antitoxic substance and if so what is its nature? The chief chemical constituent of nuclein is a substance called nucleum. Have the nucleins in general or as a class any germicidal action? As methods of isolating the nucleins are known this question can be answered by experimentation."

Forty years of research have but confirmed the theoretical foundations of 1890. That the reactions of immunity are functions of the physics and chemistry of protein materials that acquired immunity of a host to a pathogenic organism or toxic protein is in some way mediated through these same agents of offense. Vaughan's contribution to theory seems to have been a most rational and brilliant conception. It narrowed the field of research to a single group of chemical compounds however multitudinous its varieties—those basically nuclein in composition. It was easy to believe that the class of chemical compounds specifically charged with vital reactions without which there was no life should furnish both the agents of attack and defense in infectious diseases.

It was toward the end of a year's intensive work upon the vital chemistry of the nucleins with the indispensable and brilliant cooperation of Novy

and McClintock, that Vaughan, flushed with the marvellous promise of his results came in 1893 to deliver his presidential address before the section on Medicine of the Pan-American Medical Congress on "The Principles of Immunity and Cure in Infectious Diseases." Even so, his scientific inhibitions were notable in his preliminary reservation "The value of a theory does not wholly depend upon its truth, but is rather measured by the fruitfulness of the lines of investigation that it opens. Indeed, a theory may be wholly erroneous and yet it may lead to important discoveries."⁹

Due credit was given for the origin of the nuclein conception to that great trio of investigators, Bruegel, Kitasato and Wassermann. These workers, however, had found no evidence of germicidal or immunologic properties in nuclein preparations from pus. Vaughan attributed their failure to the unfortunate selection of pus as a source of nuclein and also to the drastic chemical methods for its isolation.

The researches of Vaughan and his colleagues followed a logical order. They studied through plate cultures the germicidal properties of nucleins isolated by them from various sources, as the testes, the thyroid gland and yeast cells.¹⁰

Vaughan's optimistic estimate of their results was, "Now that we have learned that the animal body itself generates a germicide more powerful in its action than corrosive sublimate, and since we know how to increase the amount of this substance in the blood, and can isolate it, and inject it into other animals, renewed hope comes to us."¹¹

It will be remembered that at about this time immunologists were divided into two camps according to their views of the anatomic seat of immunity. According to one group it was an exclusive property of cells, fixed or at large. The other group claimed that immunity was essentially humoral, a property of the blood and lymph.

It had already been discovered that the blood itself possessed definite germicidal powers. A review of the literature made it obvious that this property pertained to some proteid constituent.

Vaughan and McClintock sought to identify this proteid and formulated two questions: "Is there a nuclein in the blood serum? Has this nuclein, if there be one, germicidal properties?" Suffice it to say, they extracted from the living blood a substance which possessed the characters of nuclein and solutions of this substance proved to have distinct antiseptic if not germicidal powers. They concluded that normal blood owes its germicidal powers to nuclein.¹² This work was confirmed in a paper published by Kossel in February, 1894.

⁹Vaughan V C, Novell F G, McClintock C T. The Germicidal Properties of Nucleins. *Med News* 1893. lxiii 536.

¹¹Vaughan V C, McClintock C T. The Nature of the Germicidal Constituent of the Blood. *Med News* 1893. lxiii 701.

¹²The same thought is attributed to John Wilkins, a leading member of that coterie in 1648 which later formed the nucleus of the Royal Society. After a futile effort to develop a method of perpetual motion he reflects, "though we do not attain to the effecting of this particular yet our searching after it may discover so many other excellent subtilties as shall abundantly recompense the labour of our enquiry." *Science News Letter* 1930. p. 252.

Closely following or attending these researches in vitro many coordinate experiments were made in vivo

In his paper on *The Treatment of Tuberculosis with Yeast Nuclein*, Vaughan summarized the conclusions already noted here as well as some from experiments on animals, as follows: "3, Rabbits and guinea pigs may be protected against virulent cultures of the diplococcus of pneumonia by previous treatment with hypodermic injections of a solution of yeast nuclein. 4 The immunity thus secured is not due to the action of the nuclein as a germicide directly. 5 The process of securing this immunity is an educational one and most probably depends upon the stimulating effect of the nuclein upon some organ whose function it is to protect the body against bacterial invasion. 8, Attempts to render guinea pigs immune to tuberculosis by methods so far employed show that previous treatments with nuclein retard, but in the majority of cases do not prevent, the development of tuberculosis from subsequent inoculations. 9, Attempts to arrest tuberculosis already developed in guinea pigs by treatment with solutions of yeast nuclein, have been followed with varying results, depending upon the virulence of the germs used in inducing the disease, the stage of the disease when the treatment is begun and the susceptibility of the animal, especially as influenced by age. Upon this point we have bestowed much time and labor but the results have been so conflicting that I am not yet prepared to formulate any positive conclusions. 10, I have used nuclein with benefit in the treatment of indolent ulcer, tonsillitis and streptococcus diphtheria." 12

Two series of experiments were performed upon rabbits from which it was concluded (1) 'that rabbits may be rendered immune to tuberculosis by previous treatments with yeast nucleinic acid' further (2) 'that when treatment is begun within three or four days after the inoculation the development of tuberculosis in rabbits may be prevented by yeast nucleinic acid.'

His animal experiments led Vaughan to realize the importance of more completely standardizing his nuclein solutions. In the early work the percentage of nuclein in the vehicle was unknown and this was contaminated by other proteid material. He found it a mistake to administer the nuclein in strongly alkaline solution.

McClintock proved that guinea pigs inoculated with tuberculosis sputum died earlier and with more extensive disease when treated by hypodermic injections of sodium carbonate than the untreated controls.

After more satisfactory purification of the nuclein solution and with a standardized strength of one per cent of nucleinic acid treatment was ventured upon human patients with tuberculosis.

The diagnostic criterion for tuberculosis was the presence of tubercle bacilli in the sputum or urine respectively. The treatment of the pulmonary disease was by intramuscular or hypodermic injection of the nuclein solution, beginning with a small dose of 3 to 10 minims gradually increased to 80 minims the injections being repeated at intervals of one to several days.

In genitourinary cases the medium was injected directly into the bladder, and under certain conditions was given by the mouth

Vaughan gives a judicial estimate of his remedy. He writes "I am convinced, especially from my experiments on animals, that nucleinic acid, improperly used, may do harm. It acts as I have elsewhere shown, by stimulating the organs that elaborate the polynuclear corpuscles, and these may be overstimulated. Nucleinic acid fails to be of service unless these cell-forming organs respond. They may fail to respond on account of lowered vitality, or they may be paralyzed, as it were, by an excessive dose of stimulant."¹³

Sufficient evidence is not at hand to properly evaluate this remedy. The ever-growing scrap heap of artificial laboratory synthetics makes one wonder if it were not wiser that we try out more completely *this* synthetic discovered, manufactured and found indispensable by Nature herself.

In the middle of the '90's Vaughan had referred to me patients for treatment while they were receiving the climatic benefit of sojourn in Colorado. No adverse criticism of the remedy per se could be made, except that on one occasion I gained my first experience with anaphylaxis. A female patient had received, subcutaneously, a series of injections with apparently good effect when, one day within a minute or so of the treatment she began to suffer urgent air hunger which kept her, and myself, in great distress for perhaps an hour. Nevertheless, the experience was not without educative value, it had been habitual for me to observe the bulla on the skin caused by the subcutaneous retention of the 2 or 3 cc of solution injected. On the occasion described *no* bulla was formed, which probably meant that the fluid had directly entered the blood stream through a vein—the ideal condition for anaphylactic shock.

After two and a half years, ending December, 1895, in the treatment of tuberculosis with yeast nuclein, Vaughan summarized his experience as follows¹⁴ "The cases include all in which tubercle bacilli were found. There was no selection of cases and no exclusion. Many were in the last stages of disease when treatment was begun. I tried to carry on the treatment as if in a laboratory experiment, not to deceive myself. Of the 76 cases reported, 70 were of Pulmonary Tuberculosis. Of these 30 (42½ per cent) have died, of these at least 9 were temporarily benefited. Of the 70 cases, 17 (24½ per cent) have been continuously free from the bacillus for from one month to 2½ years so far as sputum, when present, shows. Twenty cases (28½ per cent) were still infected at the last examination, but apparently 16 of these have been improved by the treatment. Of the 5 cases of urinary tuberculosis 4 have apparently been cured, one, benefited temporarily, died of military tuberculosis. The one case of joint tuberculosis has been benefited."

The fertile mind of Vaughan produced unceasingly. From 1875 to 1914, inclusive, 198 titles appeared under his name, several of considerable volume

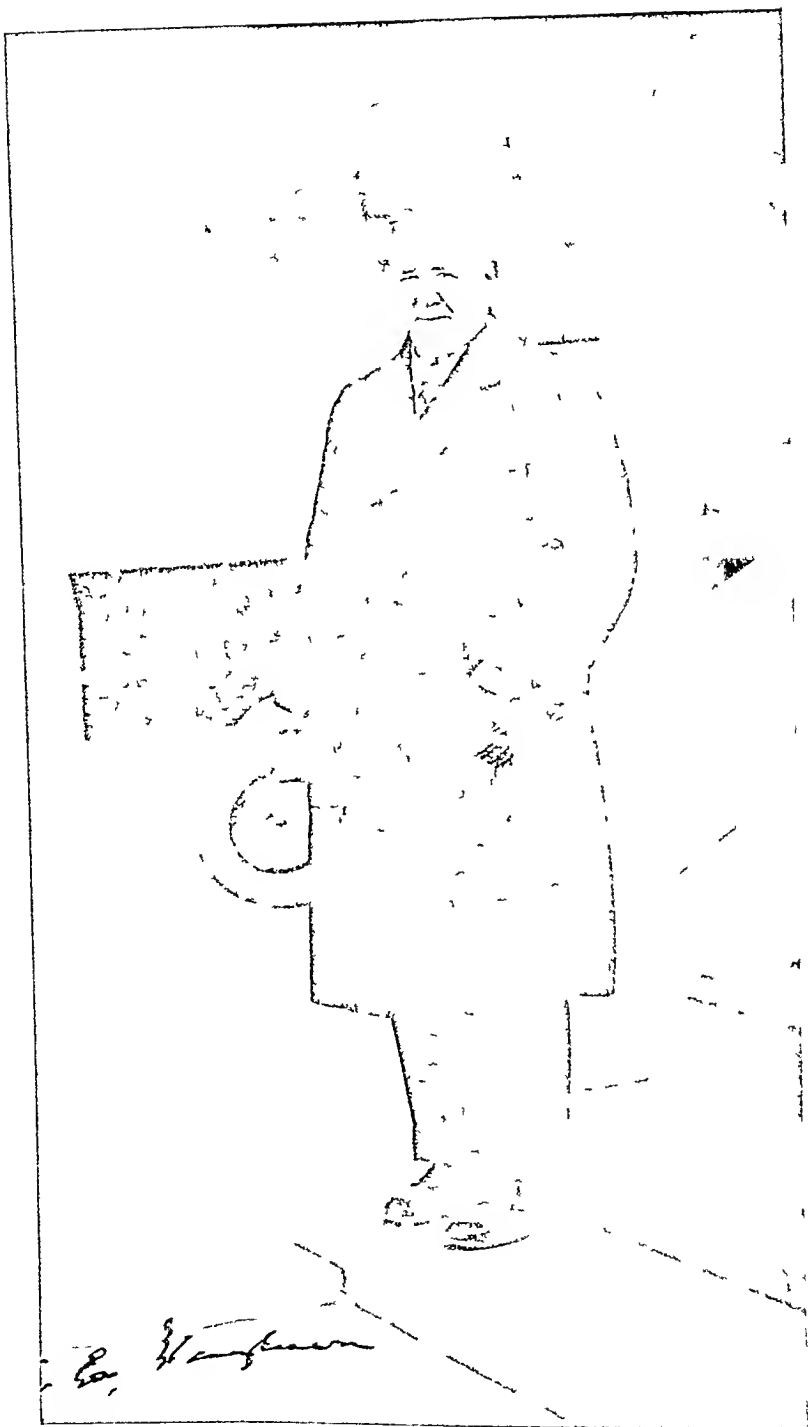
¹³Ibid p 681

¹⁴The Physiological Action and Therapeutic Uses of Yeast-Nucleinic Acid with Special Reference to its Employment in Tuberculosis. Med News N Y 1897 lxx pp 257 296 323 362 387

Equipped with intellectual and virile power as he was, the affections of the heart ruled his life through that Companion ' whose unfailing love ' he says, near the end, "has cheered me in both fair and foul weather and whose wise counsel has been my staff and support along the way "

When faltering under the final cruel blow to his health, he attended the meeting of a beloved Scientific Society in Washington to receive its gold medal of appreciation to encouraging words of a friend meant to cheer him the response was a wistful look and, It is a beautiful world "

—*Henry Seuall*
Denver



VICTOR C VAUGHAN, M D , P H D , S C D , L L D , AT PEKING, CHINA, 1927

IN GOING over the scientific papers of Victor Vaughan in chronological order, it is interesting to find that his life covered almost the entire period of the development of immunology. The book which he wrote with Novy on *Cellular Toxins* was first published in 1888. It was only ten years before this that Pasteur and Koch were carrying out their investigations on wound infections. Metchnikoff's studies on phagocytosis were not seriously begun until 1880. Pasteur's method of immunization against rabies was not published until 1885. Behring's fundamental antitoxin investigations were not to appear until 1890, and Pfeiffer's discovery of bacteriolytic and bactericidal effects were still six years in the future. Vaughan was working at a time when the conceptions of bacterial toxemia were dominated by the belief that the poisons involved in infectious disease were produced by the putrefactive and fermentative actions of the bacteria upon the proteins of the host. Brieger had published his study of the ptomaines in 1885 and Gautier, Griffiths and others were investigating the physiologic action of toxic protein derivatives in animals. Selmi in 1885 had described poisonous bases obtained from human cadavers. The work of Vaughan and Novy takes a worthy place next to these important biochemical investigations. They studied not only the ptomaines themselves but sought for similar substances in cultures of pathogenic microorganisms. Among these were the poisonous substances developed in cultures of the intestinal bacteria from summer diarrheas in infants, a subject in which, perhaps such products of bacterial cleavage may still play an important role. They isolated from mixed cultures of typhoid stools a poisonous base which was obtained as a crystalline salt and which produced purging and temperature elevations in cats and dogs. Similar substances were obtained from hog cholera cultures and from a number of other bacteria. This work logically led to biochemical investigations of food poisons, studies which contributed not only to a better understanding of the toxic protein derivatives themselves but had an important and beneficial effect upon the development of methods of food preservation.

Though the fundamental observations of these early studies were almost entirely biochemical in nature, their bearing upon infectious disease was considerable. Although their importance in connection with the pathogenicity of infectious microorganisms was diminished by the discovery of the bacterial exotoxins yet much accurate information was added to a field of protein chemistry which has become of great significance in other directions. Vaughan was quick to recognize the value of the discoveries of other men and their modifying influence upon older conceptions and in the 1902 edition of *Cellular Toxins* he incorporated a treatise on antibodies and immunity in which he demonstrated his capacity for clear reasoning—equalled, it seems to us, at that time only by Behring himself—in recognizing the necessity for

the correlation of the humoral and cellular schools of immunity. His critical comments in the summarizing chapter of this edition, dealing with this and other problems of equal importance, were entirely free from the tendency of partisanship which dominated much of the controversial literature of that period. Many of the statements and comments which he makes in this chapter, as pure deduction, especially in regard to the relations of the cellular and humoral mechanisms, have been justified by later experimental developments.

Among other things, Vaughan occupied himself at this time with a discussion of the origin of antibodies from the antigen, the Buchner retention idea which is again becoming prominent after many years during which it has been completely disregarded. That Vaughan was not easily carried away by attractive results published upon insufficient experimental evidence is apparent in his brutally frank criticisms of the results of Smirnow, who claimed at this time that he had converted toxin into antitoxin by the long-continued action of electric currents—results which had been confirmed by Bolton and Pease and others.

Vaughan's advantage over most workers in the biologic field for that day—and, for that matter, over many workers of our own time—was the broad scope of his knowledge, which covered the methods of organic and biochemistry as well as those of physiology.

His subsequent interest in the toxic split products of bacteria was a natural consequence of his earlier work. Those who professionally followed the interesting period in immunology during which anaphylactic phenomena brought a new and deeper understanding of the physiology of reactions to foreign proteins will recall the important effect of the publications of Vaughan and his collaborators on this subject. Wolf-Eisner, Friedberger and others had, at this time, brought forward the idea that many of the phenomena of bacterial injury and possibly those of anaphylaxis were due to cleavage exerted by specific antibodies upon the bacterial protein. This conception was attractive because of its simplicity and was supported by a considerable volume of suggestive experimentation. Subsequent demonstration that there was no protein cleavage involved in antibody reactions, that symptoms similar to those supposed to be due to bacterial cleavage could be produced in guinea pigs with indifferent materials, and that the anaphylactic reaction was a cellular rather than a humoral process, rendered these simple explanations of anaphylactic phenomena untenable. Vaughan's work, however, in its demonstration of a toxic constituent, chemically separable from the bacterial body, with which acute death could be produced in guinea pigs similar symptomatically and physiologically to anaphylactic death, had an important influence upon the development of what is now known as the "anaphylatoxin" reaction. This phenomenon was subsequently studied in great detail by his pupils Novy and de Kruif, and though it has shed relatively little light upon anaphylaxis, it has had great influence in elucidating a group of occurrences which one cannot help but believe possess an importance not yet fully appreciated in the pathology of the infectious diseases.

The formidable collection of papers on split products of bacterial protein were summarized in the book Vaughan published with his sons in 1913. This

volume in addition to the report of the investigations mentioned includes his experiments and ideas upon protein fever—in connection with which, incidentally, he asserted the frequent unchanged absorption of foreign proteins through the intestinal mucosa—a fact which has now become an important premise in our knowledge of the development of idiosyncrasies. This book includes, as well a thoughtful treatise on immunologic theory in which he again applied his well balanced judgment to controversial points recognizing the theories of Ehrlich as important scaffoldings for experiment but not allowing them to dominate his conceptions of immunity to the extent to which they had done this in the minds of many other leaders in this subject.

While thus, Victor Vaughan's contributions to immunology were fundamental and made him throughout his life one of the most distinguished workers in this science in America, he at the same time kept in touch with all important phases of medical development. In each of the many marginal activities which in the case of other men might have become dilettantisms or hobbies he attained eminence and made useful contributions. His extraordinary versatility can be appreciated only by an examination from year to year of his publications. During the time that he was doing the work discussed in the preceding paragraphs, largely a result of his predominant interest in chemistry, he turned his attention to problems of nutrition and the values of foods. At the same time he made excursions into the fields of pharmacology and physiology studying the physiologic action of drugs and problems of gastric secretion. And his studies on the distribution of metallic poisons in the body led him into medico legal work in which he was a pioneer in this country.

One has the impression, in reviewing his various activities, that all of them sprang logically one from another out of a superabundance of intellectual vitality and natural interest. His research appeared to satisfy only a part of his desire for action. His interest in men and affairs was expressed in the administrative activities by which he founded and guided one of the most important medical schools in America and gathered about him a faculty of distinguished scholars. And from the same interest in men generally arose his enthusiasm for public health. In this field again, both as a military surgeon in the Spanish and the World Wars, as editor of a public health journal and author of a standard work on epidemiology he transformed what might have been a purely secondary interest into a career which might have been a sufficient life work for many men. Among his purely incidental contributions in this field was the discovery of the indirect transmission of typhoid fever by flies, so important in rural and military sanitation.

It is difficult to appraise whether Victor Vaughan was more important to medicine as an administrator, a toxicologist, a medico legal expert, a biochemist, a sanitarian or an immunologist. It is useless to endeavor to do so. He made the type of contribution to immunology which kept it rigidly in the field of controlled experiment permitting himself speculation only on the basis of thorough correlation of the various fields of biologic research of which he was a master, and he influenced immunology in the United States

and in other countries by important contributions both of observation and of reasoning. And this he accomplished without in any way limiting his field and, at the same time, impressing his personality on other branches of medicine in a manner that made him a unique figure in American science. It is questionable whether, in the growing intricacy of medical investigation, activities so diversified can again be successfully combined in the lives of other individuals. In the period during which Vaughan lived, when American medicine went through its most vigorous years of growth, there was probably no other figure in the United States, with the exception of Welch of Baltimore, who had as much influence upon sound development, upon accuracy of method and upon enthusiasm for the revelation of truth as Victor Vaughan.

Of the influence of his personality on younger men both in scientific matters and in human intercourse, it is quite impossible to speak in an article as brief as this.

—Hans Zinsser
Boston

Editor's Note. Dr. Vaughan served until his death on the advisory editorial board of the *Journal of Immunology* and of the *Zeitschrift für Immunitätsforschung und Experimentelle Therapie*.

CONTRIBUTIONS TO EPIDEMIOLOGY AND PUBLIC HEALTH

VICTOR C VAUGHAN, like so many other scientists who pioneered in public health and epidemiology was attracted into these new fields of medicine through his love of chemistry

Following his introduction to chemistry in Missouri it was natural that the student should crave an appointment to the laboratory of one well versed in chemical science and so, enticed by the first edition of Douglas and Prescott's *Qualitative Analysis*, the young teacher chose the University of Michigan as a place for further education since here at least science found equal favor with the classics

Doctor Vaughan was a pioneer in public health in Michigan. Preparation not only in the laboratories at Ann Arbor but fortification with studies abroad soon brought him abreast with the newest thought and discoveries in physiologic chemistry and bacteriology. In 1888 he studied in Koch's laboratory and received instruction under the trained guidance of Carl Fankel. In that same year he visited with Pasteur and Roux, spent some days with Pettenkofer, the brilliant young epidemiologist of Munich, who had driven typhoid fever from that city, visited and studied the work of other European scholars, who, at that time, were creating the new science of bacteriology. In the early nineties he attended the International Congress on Hygiene at Budapest and heard von Behring read his paper on diphtheria antitoxin. He brought some of this marvelous curative agent home with him, some of the first in America, the very first available in Michigan.

Doctor Vaughan was a practitioner of medicine for over twenty years. During this time he not only served as the family counsellor for his many friends on the faculty at Ann Arbor and for the students enrolled at the University but he likewise had a large consultation practice throughout Michigan and the neighboring states. Thus he built up as a background for his epidemiologic studies an intimate and fundamental foundation of clinical medicine. Through his work as director of the hygienic laboratory and as a member of the State Board of Health he possessed an ideal opportunity to bring together the viewpoints of the health officer, the vital statistician, the chemist, the bacteriologist and the clinician. Doctor Vaughan described an epidemiologist as a student of etiology, symptomology and pathology. No definition could better apply to his own training. He believed that every case of infectious disease constituted potentially the seed from which many cases might develop. He not only looked upon each patient as a sick individual requiring the best of medical care but he also pictured the patient as a possible source of danger to other members of the household and the community at large. The public health aspect of the case so essential to the suppression of contagion was not wanting.

Soon after his arrival in Michigan he became interested in popular health instruction. The dissemination of scientific knowledge in terms which the

layman might readily comprehend was ever foremost in his mind as evidenced by the fact that after leaving the University in 1921 he went to Chicago for a year and under the auspices of the American Medical Association started the publication of *Hygeia*, a popular health magazine

In the early eighties it was customary to hold sanitary conventions in different towns throughout the State of Michigan. In 1882 we find one at Ann Arbor of which Doctor Vaughan served as Secretary. The announcement, a copy of which we have at hand, begins with an enumeration of sanitary apparatus placed on exhibit. The program in which the layman as well as the sanitarian participated was devoted to such public health problems as ventilation, water supply, school hygiene, control of smallpox, public health law, milk, etc. These conventions which were held several times each year in different towns usually took place at the courthouse or in some other public building to which the entire community was invited. Not infrequently were the regular meetings of the State Board of Health held at the same time and place.

Doctor Vaughan was appointed a member of the State Board of Health in 1883 and served continuously until the Board was abolished in 1919. During much of this time he was its President. His early interest in food and water is evidenced by the fact that when first appointed a member of the State Board of Health he served as Chairman of two important committees, one, that on Food, Drink and Water Supply, the other on Poisons, Explosives, Chemicals, Accidents, and Special Sources of Danger to Life and Health.

During the early years of his service as a member of the State Board of Health, he was frequently called upon to investigate reports of food poisoning and to determine the cause of epidemics of typhoid fever. In those days the Board members did not serve exclusively in an advisory capacity but assisted the able Secretary, Dr. Henry B. Baker, in surveying conditions throughout the State which might be inimical to health. In this way investigations were made of the sanitary condition of jails and public institutions where special attention was directed to an examination of the water and air supply and the means of disposing of sewage.

The State Board of Health was especially interested in stimulating research in sanitary science. At a meeting held in December, 1883, a resolution was passed that a sum not exceeding \$300 be appropriated to pay for results of original investigation in sanitary subjects and further that the Board desired to encourage special investigations into sanitary conditions at localities, with special reference to water supply, ventilation of public buildings, and the origin of epidemics of diphtheria and other contagious diseases. At this same meeting Doctor Vaughan spoke of the need of a fully equipped hygienic laboratory at the University of Michigan.

Appreciating the value of directing health education through the public schools, the Michigan State Board of Health at an early date created a Committee on Textbooks which prepared a primer on hygiene and physiology which was used very extensively. Doctor Vaughan became a member of this committee in 1883 and thus played an intimate part in popular health instruction in Michigan. Through such textbooks which were used extensively throughout the school system, the average Michigander soon became ac-

quainted with the fundamental facts concerning hygiene and disease prevention. This was to prove of inestimable value in the campaign against ignorance and superstition which contributed so greatly to the reduction of the mortality from tuberculosis and other communicable diseases.

In 1883, Doctor Vaughan became a member of the American Public Health Association which organization although but ten years old at that time, has become a leader in the promotion of public health administration throughout the North American Continent, and encouraged and fostered administrative research and studies in epidemiology and the evaluation of public health procedure. The laboratory method of thoughtful and painstaking inquiry manifested itself at an early stage in the development of the Association.

Stimulated by the probable visitation of cholera in the near future' the Association at its annual meeting in St. Louis in 1884 created a committee 'to examine the subject of disinfectants, antiseptics and germicides in their relation to preventive medicine and sanitation and to formulate a table of these agents for the information of those interested, the agents to be classified so far as may be deemed advisable according to their specific virtues, facilities of application and economy of use.'

Doctor Vaughan then a member of the Michigan State Board of Health, served as a member of this committee of which Major George M. Sternberg later Surgeon General of the Army was Chairman. The committee consisting of seven members was immediately subdivided into two groups, the first to examine the literature of disinfectants and abstract and tabulate the results and to investigate in an exact manner in the laboratory the relative germicidal value of the various substances used as disinfectants. The second group, to which Doctor Vaughan belonged was appointed especially to investigate the practical application of such disinfectants as are found efficient upon a large scale, their cost, methods of use, chemical relations, effects upon furniture or fabrics or their possible poisonous effects upon human beings or animals. Vaughan made a special study of the possible use of mineral acids including hydrochloric, sulphuric, nitrous, nitric, chromic and osmic acids. He also studied experimentally the possibility of employing mercuric chloride as a disinfectant for cesspools and privy vaults and the passage of this highly poisonous salt through the soil into wells. The study included the action of mercuric chloride on lead pipes. This committee not only reported in detail upon the numerous experiments which were conducted to determine the relative value of the known germicides but it prepared a statement of the object of disinfection and its application to sewage, excreta, clothing and general treatment of the sickroom.

Mr. Henry Lomb, of Rochester, N. Y. through the American Public Health Association offered four prizes for essays on health subjects. Dr. Vaughan's contribution 'Healthy Homes and Foods for the Working Classes,' was one of the prize winners. Published in 1886 the pamphlet of 62 pages was distributed generously throughout the country.

In October 1886, at the suggestion of the Michigan State Board of Health of which Doctor Vaughan was a member, the Regents of the University of Michigan asked the legislature for an appropriation to build and equip a

laboratory of practical hygiene in which original investigations as to the causation and nature of disease might be made. In June, 1887, the Regents established a Department of Hygiene and appointed Doctor Vaughan director of the laboratory and professor of hygiene, and Frederick G. Novy instructor in hygiene. Although the new laboratory building was not ready for occupancy until the fall of 1888, a year earlier Vaughan and Novy began their work, using rooms and apparatus belonging to the Chemical Laboratory. The first few weeks were spent in investigating fatal cases of milk poisoning. In 1885, Vaughan had succeeded in isolating the active agent of poisonous cheese, to which he gave the name tyrotoxin.

The greater part of the first three months was devoted to an investigation of an epidemic of typhoid fever at Iron Mountain. At that time there were on an average about 1,000 deaths and 10,000 cases of sickness from this disease annually in Michigan. Epidemiologic studies carried on by the new Department of Hygiene not only in the laboratory but in the field and at the bedside, called attention to the need of improving public water supplies. The dangers from polluted food supplies and the necessity of disinfecting all discharges from the patient were likewise emphasized. The nature of the poison in ice cream, cheese and other food products which produced outbreaks of food poisoning was given much attention. That the desirable diet for healthy individuals was not overlooked is indicated by the publication by Doctor Vaughan of model diet tables which appeared in the annual report of the Michigan State Board of Health for the year 1889.

The objects of the new Hygienic Laboratory at Ann Arbor were threefold, first, original investigation into the causation of disease, second, the examination of samples of food and drink, at a nominal cost, on the request of local health officers and, third, the instruction of students in hygienic investigations. Therefore health officers frequently called upon the director of the laboratory to decide as to the potability of samples of drinking water. Some analyses were made for those residing in other states. Vaughan felt that to be of service in preventing the further spread of typhoid fever, the report must be returned to the health officer within a week, at most, after the water had been received. The laboratory in its early days worked out a plan of procedure which became known as the Michigan method.

"As soon as the water is received, plate cultures are made and a test tube of beef tea is inoculated with one drop of the water. This tube is placed in an incubator and kept at 37° C for twenty-four hours. Then twenty drops of the beef-tea culture are injected with a sterilized syringe into the abdominal cavity of a white rat or guinea pig. In some instances rabbits have been used, and with these the amount injected has varied from twenty to sixty drops, according to the size of the animal. If the water contains a pathogenic germ the animal dies, usually within twelve hours. A postmortem is made, the gross appearance noted and plates are prepared from the spleen, liver, kidney, and sometimes from the blood. After twenty-four hours longer these plates have generally developed (in some instances a longer time is required), and they are compared

with the plates made directly from the water. On the plates made directly from the water the germs are counted and their general appearance noted after twenty-four, forty-eight, and seventy-two hours. In the meantime the chemical analysis is completed, and under favorable circumstances the report can be made three days after the water has been received. A week furnishes all the time required in any case. The germs taken from the plates can then be studied at leisure."

Doctor Vaughan specified as a second condition of a good report especially when the water is condemned a statement of such a nature that it would convince the average mind. He believed that chemical and bacteriologic methods then employed would not suffice and although it is true that the fact that a germ kills a rat does not furnish positive proof that it will cause disease in man it was the most convincing proof that the water was not safe to drink.

The epidemiologist and health officer of 1888 had much difficulty in convincing the layman that the polluted water which he and his grandparents had drunk for years was the cause of typhoid fever in his home. Doctor Vaughan frequently told the story of the well used by his neighbors, the water from which he declared unsafe after several cases of typhoid had developed in the household, and how he had boarded and nailed up the opening and removed the pump handle only to find afterward that another opening had been cut in the boards and a new pump provided with the inevitable result that there was more typhoid. Indeed, he stated that this persistent obstinacy and foolhardiness of the townspeople enabled Doctor Vaughan to provide for his growing family since he cared for the sick as family physician in addition to giving advice to the healthy with respect to disease prevention.

Following his service as Division Surgeon in Cuba during the Spanish American War Doctor Vaughan together with Major Walter Reed and Major E. O. Shakespeare was appointed on a board to study the causes and spread of typhoid fever among the troops in the various camps within the United States. Typhoid fever appeared in every regiment in the United States service in 1898, more than 90 per cent of the volunteer regiments being invaded by this disease within eight weeks after assembly in camp. Not only did typhoid fever appear in every regiment but became epidemic in both small and large encampments irrespective of whether the camps were located in northern or southern states. The investigations of the typhoid fever commission brought to light many facts with respect to the epidemiology of this disease which were destined to affect favorably the health of troops in future wars and materially assist in the gradual elimination of this disease from civil populations. The miasmatic theory of the origin of typhoid fever was not supported by the investigations. Murchison's pythogenic theory that typhoid fever might be generated independently of a previous case by fermentation of fecal and perhaps other forms of organic matter was found to be erroneous. The investigations of the commission did confirm the doctrine of the specific origin of typhoid fever.

Among the conclusions drawn from this study which is probably the most

complete epidemiologic study ever made of typhoid fever, the following are worthy of particular emphasis (1) typhoid fever is disseminated by the transference of the excretions of an infected individual to the alimentary canal of others, (2) this disease is more likely to become epidemic in camps than in civil life because of the greater difficulty of disposing of the excretions from the human body, (3) a man infected with typhoid fever may scatter the infection in every latrine of a regiment before the disease is recognized in himself, (4) camp pollution was the greatest sanitary sin committed by the troops in 1898, (5) some camps were unwisely located, (6) in some instances the space allotted the regiments was inadequate, (7) many commands were allowed to remain on one site too long, (8) requests for change in location made by medical officers were not always granted, (9) superior line officers cannot be held altogether blameless for the insanitary condition of the camps, (10) greater authority should be given medical officers in matters relating to the hygiene of camps, (11) in a general way the number of cases of typhoid fever in the different camps varied with the method of disposing of excretions, (12) the tub system of disposal was condemned, (13) the pit system was unsatisfactory in permanent camps, (14) the water carriage system was recommended for permanent camps and where impractical all fecal matter should be disinfected and then carted away from camp, (15) infected water was not an important factor in the spread of typhoid fever, (16) flies undoubtedly served as carriers of infection, (17) men transported infected material on their persons or in their clothing, and thus disseminated the disease, (18) personal contact was undoubtedly one of the means by which the infection was spread, (19) it is probable that the infection was disseminated to some extent through the air in the form of dust, (20) a command badly infected with typhoid fever does not lose the infection by simply changing location, (21) when a command badly infected with typhoid fever changes its location, it carries the specific agents of the disease in the bodies of the men, in their clothing, bedding and tentage, (22) after a command becomes badly infected with typhoid, change of location, together with thorough disinfection of all clothing, bedding and tentage, is necessary, (23) even an ocean voyage does not relieve an infected command of disease infection, (24) except in cases of most urgent military necessity one command should not be located upon a site recently vacated by another, (25) the fact that a command expects to change its location does not justify neglect of proper policing of the ground occupied, (26) it is desirable that the soldiers' beds should be raised from the ground, (27) in some of the encampments the tents were too much crowded, (28) medical officers should insist that soldiers remove their outer clothing at night when the exigencies of the situation permit, (29) malaria was not a prevalent disease among the troops that remained in the United States, (30) the percentage of deaths among cases of typhoid fever was about 75, (31) the shortest period of incubation in typhoid fever is probably something under eight days

While other general conclusions were made by the typhoid fever commission, those above mentioned will indicate the nature of the report, the completeness of its character and its inevitable influence upon future concentra-

tion of man power for military purposes. Particular attention was focused upon the fact that typhoid epidemics are not always due to water borne infection or to contaminated food supplies but that contact both direct and indirect may have a predominating influence in the spread of infection. That flies may serve as a vector in the spread of disease was proved beyond a doubt. The need of giving higher authority to medical officers was forcibly emphasized with the result that during the World War the medical officer was given a sufficient increase in rank so that the line officers were compelled to pay some attention to his suggestions. The epidemiologic report prepared by Vaughan as sole survivor of the typhoid commission, paved the way for improvements in sanitation which have virtually eliminated typhoid fever as a serious disease in military life. The Japanese adopted the recommendations of the commission in toto during the Russo Japanese War, demonstrating conclusively their validity.

During the World War Colonel Vaughan joined his colleagues in the Army where he served as one of the medical advisors to the Council of National Defense and as friend and counsellor to General Gorgas. His official assignment was to direct the Communicable Disease Division of the Surgeon General's office which task brought him into the very midst of the influenza pandemic of 1918. Reviewing the causes of respiratory diseases in army camps, he concluded that the greatest single factor in the prevalence of disease in certain camps and their absence in others was the natural susceptibility of the men. Aggravated by exposure, fatigue lack of warm clothing cold quarters by day, cold quarters and insufficient bedding by night, the susceptible recruits from the southern and western states succumbed to respiratory infection. He offers as the foremost remedial measure for future military concentrations a gradual introduction of civilians into army life. Men should be called to a semiactive reserve and become gradually accustomed to their new mode of life. This would permit of vaccination for typhoid and small pox, serve as a hardening process and allow of the removal and isolation of the sick prior to entry into the severe duties of army life. His military career is reviewed elsewhere.

Doctor Vaughan served on medical and public health boards and committees too numerous to mention. He was a member of the Hygienic Laboratory Advisory Board from the date of its inception, February 23 1903 and served continuously exactly twenty six years that is, to February 22 1929 when his resignation was regretfully accepted. He served for several years as a member of the International Health Board of The Rockefeller Foundation, which has done so much toward the eradication of hookworm and malaria and has served as a stimulating influence in the establishment and extension of whole time health organizations. He served for several years as Chairman of the Council on Public Health of the American Medical Association which Council did much to popularize health education. In 1903 rabies appeared in Michigan and at Doctor Vaughan's request, a Pasteur Institute was established at the University for the treatment of those who had been bitten by supposedly rabid dogs. For many years this was the only institution of the kind west of New York. Doctor Vaughan was a member

of the commission which examined the water supply at Chicago prior to the World's Fair and recommended that a private supply be obtained from Waukesha, Wisconsin, as the lake water at Chicago was becoming contaminated by the sewage from the city. In 1916, Doctor Vaughan was called to New York by Health Commissioner Emerson, and thus was brought in close contact with the devastating epidemic of poliomyelitis which invaded the city at that time.

As President of the American Medical Association in 1914, Doctor Vaughan gave a dissertation on public health, "The Service of Medicine to Civilization," which, together with his work, *Epidemiology and Public Health*, not only provides a compendium of knowledge, historical and present, with respect to disease prevention but contains many specific recommendations for improvement in public health administration which will be attained only in years to come. Ever mindful of the need of improving public health through personal health, he states that "if preventive medicine is to bestow on man its richest service, the time must come when every citizen will submit himself to a thorough medical examination once a year or oftener." Health departments must be manned with trained personnel and directed by executive officers distinguished for their knowledge of sanitation. There should be a national department of health with a member in the cabinet. Each state should have a hygiene laboratory equipped with able men supplied with facilities for the study of sanitary conditions and for the prosecution of scientific research. Members of the medical profession must carry on the fight against superstition and ignorance, must make the practice of medicine a violent factor in the battle to prolong life and prevent needless suffering and premature death.

Doctor Vaughan believed that the health of the masses could best be conserved by improving the practice of medicine and by stimulating a more intimate relationship between patient and medical counsellor. He thus formulated his ideas as to the needs of the medical profession: (1) each physician should have at his disposal every scientific facility essential to make a correct diagnosis, (2) should have a well selected library, (3) should have laboratory facilities and x-ray, (4) there should be a minimum of interference with the relation which has so long existed between physician and patient and which, on the whole, has been so satisfactory to both.

These desiderata can be served by the construction and maintenance of community hospitals. His long years of experience as a consultant in the many small towns of Michigan has shown Doctor Vaughan the meagerness of the tools with which the rural practitioner was forced to work. Many states are now making rapid progress in providing counties and districts with well-equipped hospitals, noteworthy among which are the Carolinas, generously assisted by The Duke Foundation.

Epidemiology and Public Health records most completely Doctor Vaughan's experiences and services in this useful branch of medical science. Space will not permit of extensive reference to his work and we shall content ourselves by quoting a portion of a review of the second volume prepared by Milton J. Rosenau and published in the *Journal of the American Medical Association*.

"The book continues to be a thoroughly reliable encyclopedia of knowledge concerning the ills of mankind. It is not only a compendium of reference, but also a readable textbook. Technical details are avoided, obtuse scientific researches are clearly epitomized. In fact the style has charm and directness and the high lights of preventive medicine are brought out with dramatic force. The story of yellow fever, the drama of rabies, the romance of typhoid fever and the history of plague are told with telling effect.

'One of the outstanding features of the book that merits special commendation is the conservative even cautious attitude which the author assumes toward many of the recent scientific advances. While judiciously critical concerning recent laboratory researches, the work is fully alive to modern progress; it is up to the minute. Vaughan does not admit unreservedly the nutritional cause of rickets and pellagra and adheres to the possibility of infection in scurvy and endemic goiter. It is refreshing to find our ignorance on many points so frankly and manfully stated; the histories of the medical sciences and the conquests of sanitation are not lessened by an acknowledgment of our limitations.

"Epidemiology has not yet been clearly defined. Vaughan's conception is broad and deep. He includes any information concerning a disease that throws direct or collateral light on its vagaries or that may be useful in prevention or even in understanding its nature. He draws a sharp distinction between epidemiology and bacteriology, insisting that the former is very much broader and embraces the latter. He emphasizes the point well known to epidemiologists that some diseases were worked out and sufficiently well known to form the basis of rules and regulations for their prevention and control long before their causes had been discovered. He cites cholera as an example and he might have added yellow fever and other infections. The historical side of each infection is illuminatingly drawn and the statistical side is illustrated with special examples taken largely from American experience.

—Henry F. Vaughan
Detroit

Editor's Note

Dr. Vaughan's ability to inspire with lasting enthusiasm was not limited to the classroom or the rostrum. Of this the careers of his five sons bear eloquent testimony. Three have been physicians and one is a Doctor of Public Health.

His eldest chose tuberculosis as his special study and at the time of his death was the director of tuberculosis activities in Detroit.

His second, a well known surgeon, has contributed to our knowledge of cancer through immunologic investigations.

The third son has espoused the classics and the romance languages in which he now holds a professorship in the University of California.

The fourth has selected Public Health as his life's work. His contributions in this field have already won him the Presidency of the American Public Health Association.

The fifth son is devoting his endeavors to allergy and clinical immunology.

Thus at least four of the lines of Dr. Vaughan's greatest interest are being followed by his sons. Dr. Zinsser has said that it is questionable whether activities so diversified can again be successfully combined in the life of a single individual. It has taken the combined efforts of five sons to continue in a modest way all of the interests that were so cruelly carried by the father.

DOCTOR VAUGHAN'S CONTRIBUTION TO PREVENTIVE MEDICINE*

TO MANY in this room and, in fact, to nearly all persons interested in public health work, the life of Victor Clarence Vaughan has been an inspiration. Born in 1851 in Missouri, Dr. Vaughan became a pioneer in sanitation and public health early in his career. He continued this work through his entire life, first a pioneer, then a student and teacher, and finally master of the science. Although Dr. Vaughan was versatile and spent many hours in allied sciences, his greatest interest was always in public health from the very beginning to the end. It was indeed a privilege to sit at the feet of the great scientist and hear his teachings in hygiene and preventive medicine. No one could listen and not be impressed. Dr. Vaughan, through his own personal efforts, and through his teachings to his thousands of pupils and followers, has probably saved and prolonged the lives of more human beings than any other one man who ever lived.

Fortunately many of the great teacher's thoughts were put in print and are now available as textbooks and reference books. One of his early writings was a prize essay on *Healthy Homes for the Working Classes*. This pamphlet was awarded the prize by the American Public Health Association in the early days of Dr. Vaughan's career. Written in plain language so that it might be understood by anyone who could read it, the little book has been of great value to the people. Several teachers of my acquaintance have used it as a text for lectures in hygiene.

Dr. Vaughan's accomplishments in preventive medicine were many and noteworthy and an account of them would take so much time that it would not seem just to attempt it at this time. A mere mention of some things done by our deceased friend, things which aroused the attention of the entire world, may be in place here.

Dr. Vaughan established, in 1887, the first laboratory of hygiene in the United States.

Before the discovery of the typhoid bacillus by Eberth, Dr. Vaughan made *Experimental Studies on the Causation of Typhoid Fever* in the same year that his hygiene laboratory was established.

Dr. Vaughan discovered and described tyrotoxinon, a poisonous substance elaborated in cheese. This important discovery was made during an investigation of an outbreak of cheese poisoning at Milan, Michigan.

Model Diets, published in 1887, was forerunner of the many treatises published since then on balanced diets and contained much of the present information, except on the subject of vitamins.

Dr. Vaughan made the first survey of drinking water in the State of Michigan. This was done before bacteriologic methods were known and the methods that had to be relied on were field and chemical laboratory methods.

*Courtesy of Director of Alumni Relations, University of Michigan

In 1887, at the International Medical Conference the etiology of diphtheria was said to be sewage and water pollution. Dr. Vaughan boldly stated his conviction at that very important conference at which the best minds in the world were present, that "diphtheria is not a filth disease but is induced by a specific poison." This remarkable statement was made before Klebs and Loeffler had made their announcement of the discovery of the diphtheria bacillus.

In August 1898 Dr. Vaughan was appointed a member of the Typhoid Commission. This Commission was appointed to investigate the unusual prevalence of typhoid and the excessive death rate from that disease among the American soldiers during the war with Spain. The other members of the Commission were Dr. Shakespeare and Dr. Reed. Drs. Shakespeare and Reed both died before the completion of the task and it was left to Dr. Vaughan to complete it. The results of his labors were finally published in two immense volumes and they established two monumental facts:

First, the fly transmission of typhoid.

Second, the direct contact spread of typhoid.

Dr. Vaughan's war record was one of accomplishment in preventive medicine and public health. He entered the service in 1898 at the time of the Spanish American War and during that service his most outstanding work was done on the typhoid commission as already described. He also did commendable work in the prevention of yellow fever during that service. He contracted the fever himself but met it bravely and fought it successfully.

During the World War his services to his country were invaluable. Established with headquarters at Washington, he had charge of the preventive medicine work in all of the camps and was in general charge of all of the epidemics. He described his experiences subsequently in a very valuable work on epidemiology.

For thirty six years of his life Dr. Vaughan was active as President of the Michigan State Board of Health. He was appointed by Governor Begole in 1883, ten years after the establishment of the Board, and remained a member under several subsequent administrations. In 1919 when the State Legislature abolished the State Board and put in its place a single commissioner with an advisory board, Dr. Vaughan no longer remained on the board. During Dr. Vaughan's service on the Board of Health, that institution grew from a small beginning to its present organization which is considered second to none in the United States. Dr. Vaughan suggested and introduced many activities and it can truthfully be said that his name stands as the foundation stone of public health work in the State of Michigan. I have given but a very brief resume of some of the works and deeds of Victor C. Vaughan. To have known him was to have loved and admired him; to have worked with him was a rare privilege. No one could come into close contact with Dr. Vaughan without realizing that he had profited by such contact. A complete compilation of his accomplishments and discoveries would be a compilation of the most noteworthy accomplishments in public health work during the past sixty years. The name of Victor C. Vaughan is a synonym for progress in public health.

—Guy L. Kiefer,
Lansing, Mich.

DR VICTOR C VAUGHAN

MEMBER OF THE ADVISORY BOARD OF THE HYGIENIC LABORATORY

FOLLOWING the creation of the Advisory Board of the Hygienic Laboratory authorized by Act of Congress, July 1, 1902, Doctor Vaughan was appointed as a member of the board February 23, 1903. His appointment was especially fitting because of the beginning public health movement of the time and because of the training and rich experience he had had as a physiologic chemist, epidemiologist, and public health official.

The unfortunate outbreaks of disease attending the Spanish-American War, the increasing menace of diseases, especially typhoid fever and yellow fever, and the rapidly developing science of bacteriology had all contributed to a new awakening of public interest in health affairs.

Under the law just mentioned the Public Health Service had been recognized in the 109th year of its existence, the Hygienic Laboratory had been given definite status in law and organized for public health research. The opportunities in this field were great, but from the standpoint of facilities these were lean years. The relative importance of investigations to be undertaken and the methods of making them, therefore, were of prime importance. It was the special province of the Advisory Board to consider these matters.

Problems of plague and hookworm infection had already engaged the Hygienic Laboratory. Systematic studies of typhoid fever next claimed attention. In this field Doctor Vaughan was especially helpful from 1906 to 1910. By reason of his broad experience in investigations of the outbreaks of this disease in army camps in 1898, he made many valuable suggestions regarding the conduct and interpretation of these studies. They were the first extensive epidemiologic investigations of typhoid fever among the civil population, and may be said to have marked a new era in the control of excreta-borne infections.

In the meantime Kastle was working in the Hygienic Laboratory on the ferments of the blood. Here again Doctor Vaughan's knowledge of biologic chemistry was requisitioned. In the extensive studies of Rosenau and Anderson on anaphylaxis, also, he was profoundly interested. This new phenomenon promised to have an important bearing on problems of immunology. Its spectacular demonstration before the Advisory Board in the spring of 1907 was the beginning of consideration of the subject by the board for a long period. Doctor Vaughan had already been investigating the split products of proteins, and had extracted from various proteins toxic substances which gave rise in animals to a symptom-complex not unlike that of typical anaphylaxis.

In this as in other subjects he advanced original hypotheses to stimulate interest and study on the part of laboratory workers. It was the custom

regularly to have members of the board address student workers in the Hygienic Laboratory. In a particularly happy manner Doctor Vaughan would present his hypotheses assuming, as he said, that his hearers were trained to consider phenomena not yet fully understood and to discriminate between proved facts and data requiring confirmation. For over twenty-five years he remained a member of the Advisory Board. During this formative period many fundamental problems received attention in the Hygienic Laboratory. These related to cholera, plague, pellagra, poliomyelitis, typhoid fever, tuberculosis, typhus fever, tularemia, and other public health matters.

In company with Professor W. H. Welch and Assistant Surgeon General J. W. Kerr he visited places in the South, including Milledgeville, Georgia, in 1917, where at the time Goldberger's epoch-making studies of pellagra were being conducted. By these personal inspections it was possible not only to suggest additional methods but to verify progress already made.

In all the years of his association with the Hygienic Laboratory he rarely failed to attend a meeting of the Advisory Board. Notwithstanding the great demands upon his time and energies as teacher and research worker in his own institution, he readily responded to orders convening meetings of the board.

For these important services only nominal compensation could be allowed. Under law it is impossible to do so adequately. Doctor Vaughan's long tenure of office under these conditions is but another evidence of the willingness of men of eminence to render public service without thought of remuneration other than the personal satisfaction in the good accomplished. He was in the highest degree a public servant. He was more. By reason of his scientific attainments he was an invaluable advisor and because of his character he was an inspiration to younger men working for the public good. As a friend he endeared himself to those immediately responsible for the development of public health research—a major function of the Federal Government.

—*Hugh S. Cumming,*
Washington, D. C.

A FIGHTER FOR THE CAUSE OF HEALTH

THE MILITARY RECORD OF COLONEL VICTOR C VAUGHAN

DR VICTOR C VAUGHAN in his delightful autobiography *A Doctor's Memories* remarks, "My life has been determined by heredity and environment" His grandfather, William Dameron, saw service in the Black Hawk War with the rank of Colonel His father, John Vaughan, for a time served in the Regular Army Four years of the most impressionable period of Doctor Vaughan's childhood were spent amidst scenes of internecine strife in Missouri during the Civil War, which taught him, in his own words, "to hate war and to love peace so dearly that I have been willing to do my small bit of fighting for it" From this ancestry and environment Vaughan received the impulses which directed his career along many lines of military activity, all of which were calculated to ameliorate the horrors of warfare The hereditary trend was passed on to his sons, all five of whom served as commissioned officers during the World War, three going to France The eldest, Major Victor C Vaughan, Jr, Medical Corps, lost his life by drowning in France after the Armistice

Having graduated in medicine, having filled important positions in the University of Michigan, and while dean of the Medical School, Doctor Vaughan left the scholastic quiet to do his share during the Spanish-American War in 1898 After the proclamation of war he was at the first anxious to enlist His eagerness for service is shown by a yellowed sheet in the files of the Surgeon-General's office, which reads as follows

"University of Michigan
Department of Medicine and Surgery
Ann Arbor, March 26, 1898
Geo M Sternberg, M D, LL D,
Surgeon General, U S A
Dear Dr
If there be any need of volunteers for the Medical Service and I am not too old, I wish to be counted in
I suppose that you have your hands full just now
Yours
V C Vaughan "

Immediately following a patriotic speech made before the students of the University of Michigan, he was appointed by Governor Pingree on June 9, 1898, as Surgeon of the Thirty-Third Michigan Volunteer Infantry with rank of Major, and was mustered in the same day He proceeded to Camp Alger, Va, for duty, sailing with his command for Cuba a few days later and landing

near Siboney The boat in which he disembarked capsized when approaching shore and the occupants reached land by swimming Major Vaughan shared this unpleasant experience with his college associate, Major Charles B G de Nancrede, Division Surgeon

On July 1 1898, Major Vaughan was with a command suffering several casualties which necessitated the application of first aid dressings under fire His efficient services in this situation were specially mentioned by his brigade commander, Brig Gen Henry M Duffield as a result of which he received a citation (Silver Star) for gallantry in action under date of September 17, 1924 As Major Vaughan says in his *Memories*, Uncle Sam may be slow in conferring honors, but he seldom wholly forgets

While at Siboney Major Vaughan suffered from a very serious attack of yellow fever and for a time his life was despaired of Soon after his recovery he returned to the United States via Tampa, Fla, on duty with convalescents, later proceeding in charge of patients to New York Under date of July 8 1898, he had been appointed Major and Division Surgeon, U S Volunteers On arrival in New York he received orders to report to the Surgeon General in Washington for conference regarding the duty prescribed by the following order

' War Department
Adjutant General's Office,
Washington August 18, 1898

'Special Orders }
No 194 }

"49 A board of medical officers, to consist of Maj Walter Reed, surgeon, U S Army Maj Victor C Vaughan division surgeon U S Volunteers and Maj Edward O Shakespeare brigade surgeon U S Volunteers is appointed to meet in this city at the earliest date practicable for the purpose of making an investigation into the cause of the extensive prevalence of typhoid fever in the various military camps within the limits of the United States, under such instructions as it may receive from the Surgeon General of the Army The board will call the attention of the proper commanding officers to any insanitary conditions which may exist at the camps visited by it and will make recommendations with a view to their proper correction The report of the board will be forwarded to the Surgeon General as soon as practicable after the completion of the investigation contemplated

' Such journeys as may be required under the above order are necessary for the public service

' By order of the Secretary of War

' H C Corbin

' Adjutant General '

In carrying out these instructions the board visited the following camps on the dates stated August 20, Camp Alger at Dunn Loring Va August 26, camp at Fernandina Fla August 28 Camp Cuba Libre at Jacksonville, Fla September 7, camp at Huntsville Ala September 10, Camp George H Thomas at Chickamauga Park Ga, September 14, camp at Knoxville, Tenn, September 30, Camp Meade, near Harrisburg Pa Careful investigations were made at these encampments along all of the lines then known to epidemiologic science Patients who had been transferred elsewhere were methodically

One of the fundamental conclusions of the board was to the effect that little malaria had occurred and that the prevailing sickness had been typhoid fever, which was either unrecognized or else diagnosed very late in its course. About 20 per cent of the men at the national encampments were thought to have suffered from this disease. In view of the prevalence of typhoid fever at that time throughout the United States, it was considered probable that nearly every regiment had brought to camp one or more cases of typhoid in the incubation or prodromal stage, and that the spread of the disease among the troops had been mainly from camp pollution—soil, latrines, blankets, clothing, tentage, etc., and by direct contact from man to man. Flies were considered to be important spreaders of infection, from the poorly kept latrines to exposed food. Water pollution was not deemed an important factor in the dissemination of the disease through these camps. The progress of typhoid infection was shown to be characterized by a series of company epidemics. In view of our present knowledge of the importance of carriers and missed cases in the spread of localized epidemics originating in the kitchen, it is interesting to note that this idea had not come into being at the date of these investigations and was not even hinted at in the report of the board.

The findings of the Reed Vaughan-Shakespeare board led to very marked improvements in our methods of handling large bodies of men in camp and of treating them in hospitals, thereby contributing materially to the success of preventive medicine before and during the World War. One conclusion of the board, since realized to a large degree, was that "greater authority should be given medical officers in questions relating to the hygiene of camps."

A preliminary abstract of the Board's work was published by the Government in 1900. Major Vaughan labored on the final assembling and analysis of the voluminous data until June 30, 1899, on which date he was honorably mustered out of the Federal service. After this he voluntarily continued to work on this subject until January 31, 1900. Major Shakespeare, whose extensive experience with epidemics of cholera and typhoid had been of great value during the investigations, died suddenly on June 1, 1900. Major Reed, whose time was much occupied with his epochal yellow fever researches, had the report in charge until his death on November 23, 1902, at which date it was still unfinished. The completion of the manuscript and supervision of its publication by the Government Printing Office in 1904 with the funds appropriated by Congress was carried out by Major Vaughan. The finished work in two large volumes entitled *Report of the Origin and Spread of Typhoid Fever in U S Military Camps During the Spanish War of 1898* is a masterpiece of painstaking analysis and bears the following words of appreciation:

"War Department,
Office of the Surgeon General,
Washington, November 1, 1904

* * * *

"Professor Vaughan, whose special fitness for this work is well known to the medical world, has labored unceasingly to put the full report in such shape as to make it a classic not only for the study of the epidemiologist, but also for the use of the entire medical profession.

"I desire to express my appreciation of the successful results of his painstaking and intelligent work

' C L Heizmann,
Assistant Surgeon General U S Army
Acting Surgeon General '

Doctor Vaughan's service in the interest of typhoid prevention did not cease with the publication of the work of the Commission. In 1908 the Medical Reserve Corps of the Army was organized by Surgeon General R M O'Reilly, and Doctor Vaughan was commissioned as 1st Lieutenant with rank from July 5, 1908 being one of the first recruits in accordance with General O'Reilly's policy of having the corps headed by some of the outstanding physicians and surgeons in the country. Following the report of Major F F Russell regarding the practice of antityphoid inoculation in Europe, Doctor Vaughan was called into active service December 5 to 12, 1908, and was detailed as a member of the board appointed to consider this report. Aside from the Surgeon General and Major F F Russell who was recorder, this board consisted of the following members of the Medical Reserve Corps: Dr Wm Councilman, Dr John H Musser, Dr Alexander Lambert, Dr Simon Flexner, and Dr Wm S Thayer. The board found that antityphoid inoculation was a safe and practicable method for diminishing the amount of typhoid both in peace and war; it recommended its use among all forces in time of war and its immediate introduction in the hospital corps and army nurse corps and among volunteers from other branches of the service. The recommendations of the board were approved and formed the basis of G O 10 War Department, 1909. A little later antityphoid inoculation was made compulsory for the entire Army and Navy. The practice of typhoid vaccination is considered to be one of the most important factors which led to the greatly reduced incidence of the disease in the Regular Army and to its relative infrequency among all classes of military personnel during the World War.

Although at the outbreak of the Spanish American War in 1898 Doctor Vaughan had raised the question as to whether he was "too old" for military duty, we find no record that this issue was again raised by him when nineteen years later, the United States embarked on its much greater venture in the World War. As in 1898 he was impatient for service, Doctor Vaughan was promptly called to the assistance of the country when war appeared imminent. During the winter of 1916-1917 when it became increasingly probable that our country would be drawn into the conflict, the National Research Council of the National Academy of Sciences, including on its roll eminent men from every branch of scientific endeavor, began to gather information which would be of use in case of an emergency. Doctor Vaughan looked after medical affairs for the Council and made frequent visits to Washington and New York being in close touch with General Gorgas and the other personnel of the Surgeon General's Office. Among the subjects considered were sterilization of drinking water, ventilation of barracks, uniform clothing rations, typhoid and smallpox vaccination, detection of disease carriers, treatment of the gassed, protection of the ears from effects of high explosives, provision of laboratories, bacteriology of wounds, and the procurement of medical supplies. Later the

National Research Council acted as the scientific component of the Council of National Defense. War was declared on April 6, 1917. Already a lieutenant in the Medical Reserve Corps since 1908, Doctor Vaughan was called to active duty April 26, 1917, having been promoted to Major on April 9, 1917. He was assigned to duty with the medical division of the Council of National Defense where he continued to be engaged with many of the problems listed above, and in addition conferred frequently with General Gorgas and served on a committee to scrutinize the qualifications of the numberless applicants for commission in the Medical Department. In August, 1917, he was definitely relieved from duty with the Council of National Defense and assigned as head of the Communicable Disease Section of the Division of Sanitation in the Surgeon-General's Office. In this position he had immediate supervision over the current reports of communicable diseases from all the camps and hospitals in the United States, compiling statistics, developing graphic charts, analyzing data, and investigating methods of disease prevention. He emphasized the fact that a much greater prevalence of respiratory diseases was found in those camps where the population was drawn largely from rural communities. With the assistance of Capt. George T. Palmer, he prepared a valuable article entitled *Communicable Diseases in the National Guard and National Army of the United States During the Six Months From September 29, 1917, to March 29, 1918*, which was published in the JOURNAL OF LABORATORY AND CLINICAL MEDICINE, Vol. III, August, 1918.

During his period of active duty, Colonel Vaughan made a great number of inspections relating to medico-military activities. In June, 1917, Col. Frederick P. Reynolds, Medical Corps, and Major Vaughan proceeded under official orders to Ottawa, Montreal, and Quebec for the purpose of reporting upon the administrative and sanitary procedures of the Medical Department of the Canadian forces. One of the most important services rendered by Colonel Vaughan had to do with the numerous sanitary inspections he made during the latter half of 1917 and the spring of 1918. On many of these journeys he accompanied General Gorgas. Measles, pneumonia, and meningitis were then causing much anxiety throughout the country. Colonel Vaughan's large experience was of much value in these troublesome times and the prestige and authority of his name assisted greatly in maintaining morale and in supporting the policies of General Gorgas. Among the points visited between September, 1917, and February, 1918, were Camps Funston, Beauregard, Pike, Bowie, Sevier, Doniphan, Wheeler, and Custer. Other inspections were made during the influenza epidemic in the fall of 1918. To quote his own words:

"I went to Camp Devens as soon as influenza was reported and the realization of the utter helplessness of man in attempts to control the spread of this disease depressed me beyond words. We are inclined to boast that the age of pestilence has passed, but, with a fair acquaintance with the history of epidemics, I dare say that the world has never before known a pestilence more widespread, more intensive and appalling in its progress, or more destructive to life, than the epidemic of influenza which apparently came into being and grew in violence as the World War passed through its final stages. It seemed

that Nature gathered together all her strength and demonstrated to man how puny and insignificant he and his forces are, with all his murderous machinery in the destruction of his fellows. We have not passed beyond the age of pestilence. Much has been done in man's struggle against disease, but greater things are to be done. There has been no armistice signed between man and disease. Influenza, pneumonia, cerebrospinal meningitis, poliomyelitis, and tuberculosis are still using weapons against which our defense is quite inadequate. They employ strategy in approach and attack which we do not fully understand. In this war against epidemic disease we must not permit the elation due to past victories to make us less careful and thorough in preparation for the battles of the future."

Between inspections Colonel Vaughan was engaged in duties connected with the study of current epidemiologic records received in the office of the Surgeon General. By par 161 Special Order 118, War Department, May 20 1918, he was made a member of a board of medical officers appointed to investigate the nature, cause, prevention and treatment of pneumonia and its complications in the camps within the United States. The other members of this board were Col Deane C Howard, Col Frederick F Russell, Col William H Welch, and Contract Surgeon Rufus Cole. During this investigation he visited Camps Gordon, Devens, and Wheeler and General Hospitals No. 6 at Fort McPherson, Ga. and No. 12 at Biltmore, S. C.

In 1922 Colonel Vaughan assisted by Henry F. Vaughan and George T. Palmer, published an extensive and notable work entitled *Epidemiology and Public Health*. The discussions regarding typhoid fever, malaria, measles, pneumonia, influenza, and cerebrospinal meningitis are to a considerable extent based upon Colonel Vaughan's experience in the Army and represent his matured thought on diseases which have played such a vital part in our wars. The chapters devoted to these subjects contain careful analyses of military experiences which should be studied by every medical officer.

Colonel Vaughan was promoted to the grade of Lieutenant Colonel with rank from February 11 1918, and to the grade of Colonel with rank from April 30, 1918. He was honorably discharged from the Federal service February 14, 1919.

In appreciation of his services to the Allied cause the Republic of France honored him with the decoration of Chevalier Legion d'Honneur.

Colonel Vaughan was a man of pleasing personality, mild-mannered yet inflexible in carrying out the policies which he considered necessary to attain his aim of preserving health. A long and honorable record, the prestige acquired through his addresses and publications and the energy displayed in prosecuting investigations together rendered his services of inestimable value to the Surgeon General during the trying period of the World War. His example of devotion to the public welfare is an inspiration to physicians both in and out of the military service. His worth to his country was recognized by the award of the Distinguished Service Medal with the following citation:

"Colonel Victor C. Vaughan, United States Army. For exceptionally

meritorious and conspicuous service During his service in the Office of the Surgeon General his contributions of advice and information have been of great value to the Army in connection with the control of communicable diseases During the recent epidemic of influenza, in particular, his work was of extreme value "

—*Merritt W Ireland,*
Washington, D C

OUR COLLEAGUE DOCTOR VICTOR CLARENCE VAUGHAN*

TO BUT few is it given to leave upon the world so strong and enduring an impress as that of our colleague, Victor Clarence Vaughan. His life curve which stretched out to near fourscore years shows almost to the end an ascending arc and one which reached to high levels of attainment.

When his more strenuous activities were already behind him, out of a rich experience, with abundant leisure, and near the libraries of the national Capitol, he began the writing of his *Epidemiology*, through which he hoped to project himself into the future. This authoritative work he was spared to complete and to see issue from the press and then with the retrospection that comes in the sunset of life while yet in full possession of his intellectual powers and with a vivid memory scarcely impaired, he set down the story of his eventful life. In his *A Doctor's Memories* with greater success than has been given to most others he was able to impart to the printed page of his life story the intensely human side of his personality and to leave with his readers a measure of the peculiar personal charm of the Vaughan we knew and loved.

And what a circle of friends he had made to read his story! There was the group of university workers, almost the entire medical profession, the army, the scientists of the government bureaus, the medico-legal fraternity and that wider circle with which he had touched elbows on his extended travels both in this country and abroad.

My colleagues upon this memorial program have already touched upon the professional life of our friend, and it has been left to me to speak of Vaughan, the man apart from the work in his chosen field.

In his married life Vaughan was peculiarly blessed, and his family circle was to him a continual source of inspiration and help. The five sons seemed to inherit the high ideals of their parents, and each carved out for himself a career of usefulness in the intellectual field, four of them in the medical profession, and in their success Vaughan had a father's joy and pride.

Hardly less deep and strong in him than the love of home was the love of country. While a boy on a Missouri farm during the Civil War between the States he early became familiar with the horrors of war and especially with the brutality and license of guerrilla bands in border warfare which culminated in the raiding of his home. An intense hatred of war was thus bred in him, but one which never blinded him to his duties as a citizen of our common country and so in both the Spanish American and the World War he promptly volunteered for active service. In 1898 both by precept and example he stirred the student body into a blaze of patriotic fervor, and as major in the medical corps of the army he served with distinction through the Santiago campaign in which he nearly succumbed to an attack of yellow fever.

meritorious and conspicuous service During his service in the Office of the Surgeon General his contributions of advice and information have been of great value to the Army in connection with the control of communicable diseases During the recent epidemic of influenza, in particular, his work was of extreme value "

—*Merritt W Ireland,*
Washington, D C

ago he was instrumental in founding. Its membership has been limited to twenty men who must be members of the faculty, and it has met fortnightly at the homes of members for social intercourse and for intimate discussion of those problems in which its members are most keenly interested. Unless absent from the city it was rare for Vaughan to miss a meeting of this club, and he brought to it a never failing good cheer, a fund of humor, and a wealth of stories out of his varied experiences. Discussion of the paper over, Deans Vaughan and Cooley became the center of an intimate exchange of views on topics of the day and especially the campus, and of anecdotes drawn from experiences in many fields. Here as nowhere else this group of his colleagues came to know and love the man of high ideals, of generous impulses, of straightforward methods, and of keen interest in his fellow workers both in the University and throughout the country. His passing from among us has left a void of which we are today keenly aware and one which is not likely to be filled.

—William H. Hobbs,
Ann Arbor, Mich

DR VICTOR C VAUGHAN AND HIS RELATIONS TO THE NATIONAL RESEARCH COUNCIL

DURING the Civil War President Abraham Lincoln stimulated the formation of the National Academy of Sciences which included the leading American men of science who gave freely of their advice on all technical matters to the President and to Congress. This body continued its scientific deliberations during the years of peace from 1863 to 1916 and was recognized as the highest court of appeal, although infrequently called on officially by the government to settle vexed scientific questions.

It became evident to most thinking men in 1916 that the United States of America was destined to be involved in the World War which was sweeping Europe. Although our government had been pledged by President Wilson to a policy of neutrality, it seemed criminal to remain deliberately unprepared for entrance into the arena of war across the seas.

The National Academy of Sciences enjoys the privilege of governmental affiliation without governmental control. As a chartered body it was free to think and to prepare itself for the inevitable which the Government officially must apparently ignore. In April, 1916, the Academy by unanimous vote tendered its services to President Wilson who at once accepted them. It took further a wise step in enlarging its selective body through a larger and more democratic subsidiary which it christened The National Research Council. This Council was designed, and has since proved to be, a clearing house for all scientific questions, both national and international, in its several divisions which cover the entire realm of science.

Dr. Vaughan, as ever far sighted and wise, was one of the first members of the Academy to bring into existence this active working body which through its representation in all national societies was best suited to find the man most fitted for each problem as it arose. He was a member of the first executive committee which made the Research Council alive to its country's needs during the trying war years of 1916-1919 and the first chairman of the Committee on Medicine and Hygiene. With characteristic energy and with his knowledge of what war could mean from the medical standpoint, Dr. Vaughan undertook to ascertain and to formulate the problems of most vital significance in the care of troops in camp and in the field. His intimate friendship with General Gorgas and other governmental officers made it possible for him to obtain their unofficial expressions of opinion as to what the mobilization of a hitherto unprecedented number of men would mean in safeguarding them from disease.

Dr. Vaughan not only formulated the vital problems at issue but at once undertook to obtain the best available advice and aid in solving them. No one was better informed as to active centers of research and authority in the

whole field of medicine in the many active university, governmental, and private laboratories. He has listed in his autobiography some of the problems needing solution as follows:

- 1, The best method of sterilizing drinking water for troops in cantonments, on the march and on the firing line
- 2 the ventilation of barracks
- 3 soldiers' clothing
- 4 rations
- 5 the best methods of vaccination against both smallpox and typhoid fever
- 6 the treatment of wounds
- 7 the treatment of poisoning with deadly gasses
- 8 the provision of supply of medicines
- 9, the protection of the ear against high explosives
- 10 the detection of disease carriers and their treatment
- 11 provision of diagnostic laboratories in both equipment and personnel and
- 12 the bacteriology of wounds

It might appear from Dr. Vaughan's recital of these problems that the regular officers of army and public health service not only appreciated these problems but knew best how to solve them. No one outside governmental service could so well understand so well the needs and responsibilities that would devolve on those in active service but the very solidarity and responsibility of these services shut them off from constant contact with the perennial fountain heads of progress in universities and civilian life where the delicate growth of research is fostered by freedom from less formalized methods of life.

The two newly organized civilian advisory bodies the National Research Council and the Council of National Defense, were in a particularly advantageous contact position between official need and responsibility and nongovernmental knowledge and research ability. No one could appreciate better than Dr. Vaughan both sides of the question no one was better fitted through charm and strength of personality to bring about the delicate adaptations which the situation demanded.

As a result of these adaptations for something like a year (1916-1917) the medical forces of the United States found themselves prepared in an amazing degree for their responsibilities when this country entered the war. It is not within our province to consider the army record of Dr. Vaughan during the World War as chief of the Division of Epidemiology all major questions of the health of our troops came under his wise surveillance. He still continued his unofficial interest in the National Research Council during its entire period of war organization 1916-1919.

When the National Research Council entered in its permanent organization in 1919 Dr. Vaughan was as ever a guide and counsellor not only in its Medical Division but in its larger relations. He remained intimately connected with it throughout the remainder of his life. When relieved of his university responsibilities in Michigan Dr. Vaughan was fortunately able to serve actively as the Chairman of the Medical Division for two different years (1921-1922 1925-1926). As the first chairman of the permanent organization he was able to shape many of the important policies which have made the medical division useful notably in the establishment of the fellowships in medicine for which he was largely responsible.

It seems fitting indeed that the last active duties of one whose life was filled with a multiplicity of personal and public services should have been in Washington at the very center of our national interest, and particularly connected with an organization destined, we believe, to become a clearing house for forwarding research both national and international in scope. It served to emphasize the productive scholarship and scientific eminence for which Dr. Vaughan will always be remembered.

—*Frederick P. Gay,*
New York

AN APPRECIATION*

APPROXIMATELY the definition of genius as 'remarkable aptitude for some special pursuit' a posthumous biographer would find himself at once and irreconcilably at variance with Dr. Vaughan's estimate of his own qualities. He writes in *A Doctor's Memories*: 'I am not a Chinaman and do not practice ancestor worship but I do respect my forebears and acknowledge my indebtedness to them. They have transmitted to me no spark of genius. I am not aware that any of them ever possessed such a gift be it in form of a blessing or a curse.'

His descent in the maternal line was from French Huguenots who came to this country in 1699 settled at first on the James River and eventually became dispersed through Virginia and North Carolina. His family so far as he could ascertain, bred constantly plain people honest according to the standards of its several generations and rebellious to dictation from others in religion, morals, and politics.

Ample confirmation of the final sentence in the preceding quotation from the *Memoirs* is furnished by an episode in his career at the University of Michigan. While the matter of promotion was pending in the Board of Regents, the charge of atheism was introduced. To Dean Palmer who in agitation revealed this and suggested the importance of denial he said: 'Tell the Board that I decline to make confession of faith to them. The position concerns the teaching of science and has no relation to religious belief.'

And resistance to coercion was natural and ingrained. An ancestor fought in the Revolution, a relative had part in the Black Hawk War, another was surgeon in the Confederate Army and his father served for a time in the United States Army.

Dr. Hubert Worl, president in 1920 of the American Medical Association, is quoted as saying: 'You all know that Dr. Vaughan is already known as the greatest man in American medicine in Michigan and a great many of us believe he is the greatest man in American medicine today.' Obviously appraisal of the values of such a life must be a composite product and can from no particular pen, however facile, appear even measurably just and complete. Much less may an estimate from the present writer, all too ill equipped for the service, fulfill requirements and be satisfying. The opportunity is welcomed, however, to pay tribute to this extraordinary man, my friend. I admired him and to employ a good old fashioned word, 'liked' him. Every confidence or suggestion he ever gave me was prized and for many, many years I felt definitely at home in his company.

My memories of Dr. Vaughan hark 'way back to the winter of '76-7 when young, verdant, and inadequately prepared I became a student in the Medical Department of the University of Michigan. He was also young—five years

*Courtesy of the Journal of the Michigan State Medical Society. J. H. Dempster, Editor.

my senior—but he had a cultural and pedagogic background. In passing, it may be mentioned that nothing whatever of this was displayed in his attitude toward students. Indeed, his sympathetic understanding and considerateness related him perhaps more closely with them than with the teaching staff of the department, all older and highly worthy men who had arrived. However, he was at that time definitely on his way to distinction.

From Mount Pleasant College, Missouri, where he was graduated in 1872 and taught Latin and chemistry until 1874, lured by Douglas and Prescott's *Qualitative Analysis* which decided in his mind the long-debated question whether to choose the classics or science for his life work, he came to the University of Michigan for postgraduate study. There he acquired in 1875 the degree of Master of Science, in the following year that of Ph.D., and in 1900 an LL.D.

He entered the Medical School in 1876 and was graduated two years later. Before matriculation therein he had acted as voluntary and temporary demonstrator in the dissecting room. His appointment as instructor in physiologic chemistry followed the enforced retirement of Professors Douglas and Rose which came about through careless business methods and was, he declares, a regrettable and sorrowful affair.

In his first appearance before the student body he tactfully avoided any subject in chemistry and spoke on "The Structure and Function of the Kidney." Potentially hostile partisans on both sides of the controversy were placated and all went out singing, "He's a Jolly Good Fellow." Commenting upon this in "*Memories*" he writes: "During the forty-five years that I continued to lecture to medical students not one has ever shown me the slightest disrespect in classroom or elsewhere."

During my brief student days in the University he was instructor in the chemical laboratory over which presided the distinguished Dr. A. B. Prescott who "with a benignant smile and a genial voice answered the students' queries both the wise and the unwise." What Dr. Vaughan thus writes was equally true of his own painstaking efforts greatly appreciated by students. There was naturally nothing which savored of intimacy between himself and them but a cordiality existed in their relationship. My own acquaintance with him, of course quite casual at the time, ripened eventually into enduring friendship and is treasured as a choice possession.

I never suspected until encountering the story in *A Doctor's Memories* that his early education had been all 'round of such a liberal character. That he was gifted as a teacher all having acquaintance with his methods can testify and it is plain that familiarity with the classics lent much to the well-chosen diction present in both his verbal and written productions.

It was the theory of President Terrill, the "greatest educator (he) ever knew," of Mount Pleasant College, where Dr. Vaughan became the "Alpha and Omega of the advanced class in Latin," that no one "knows anything until he can state it in writing."

To Dean West of Princeton, Dr. Vaughan said: "Although my adult life has been given to sciences, I wish to testify that the first author to stimulate the pyramidal cells of my cerebral cortex was old Virgil and even now in my

old age, there is only one book which I prefer to Virgil and that is Dryden's translation, which I read with less effort.' Of Professor Fieze he writes that to be with him was to receive lessons in grace and courtesy. He was my ideal of a learned man. I could not make of him a Trojan hero not even an Aeneas, he was a Virgil himself.

Concerning his old home in Missouri, colored by the imagination of Walter Scott the stately lines of Virgil and the eloquence and wisdom of the great pragmatist, Cicero he is no less than poetic. Of the vicissitudes of childhood when during the Civil War brother was arrayed against brother and where he learned to love peace so dearly that a willingness to fight for it developed, he writes thrillingly but without bitterness.

'Whatever I may intend to say he declares 'when I am to make a speech, when I actually begin to talk I always give expression to my convictions.' 'God pity the country he exclaimed in mental frenzy at a mass meeting where there was considered a call to arms for the Spanish American War—'whose tramps must fight its battles. This speech Dr. Vaughan humorously writes, brought about a commission from Governor Pingree. Some enlist because they like the soldier's life some for patriotic reasons but I received my commission because I killed too much.'

No manner of doubt exists in the minds of those who knew him well that he spoke from conviction. His language was plain and forceful. At a meeting of the State Medical Society in 1883 he said 'I have attended several meetings but never before have I known the Committee on Admissions to wait so long before reporting. There is an apparent intention at least to chafe off those who have come here to join this Society. During a symposium in the same Society in 1894 he inquired [I can hear his voice] whether there were any bacilli in those guinea pigs anywhere in those guinea pigs, when they died of tuberculosis. The one interrogated could not reply off hand. He (Vaughan) thought the logic employed in the discussion was bad that the only possibility of controlling the spread of consumption consists in the destruction of the bacillus.

Those who have been perplexed and irritated by the frequent neologisms purveyed in medical nomenclature are entitled to a chuckle over his pronouncement, the coming of new words is sometimes mistaken for progress in science. His Memories are shot through with practical humanitarian philosophy.

From early years at the University the Vaughan home was in open house for students. During forty-five years of teaching no graduate of the Medical School escaped an invitation there. His disciplinary measures toward the careless and intemperate consisted at first in a warning which betrayed acquaintance chapter and verse with the student's shortcomings. He was accustomed in classes to emphasize the danger to others through impure contacts. His 'as an individual you are of no importance anyhow risk getting venereal disease if you must was apt to be efficacious with the lustful.

His rise was rapid. In 1887 he became dean of the medical faculty. Among his choice Memories are appreciations of his sometime colleagues.

Dr Foid "knew anatomy, both human and comparative. He lived and taught it in a way that held the individual attention of every student—he awakened a love for it in his hearers."

Alonza B. Palmer was "a great teacher of internal medicine."

George E. Frothingham "was my preceptor and I cannot speak of him without love and reverence."

Macleam was a most fascinating man. "I do not think that any teacher in the University within my times was so greatly admired by the students as he."

Of Dr. Charles B. de Nanciede he writes, "I cannot overestimate the services rendered by this man to the University."

Of Dr. Darling, Dr. de Nanciede's successor, he "honored his chief and himself in a splendid way."

And of a venerable friend, "I left the cottage bearing in the memory chamber of my brain a portrait of a saint such as no old master ever painted."

When he resigned from the University, a newspaper reporter asked for a list of his discoveries. He was told that there were many important ones and was given the names of Doctors Novy, Hubei, Warthin, Edmunds, Weeler and others.

Among my pleasantest memories are those of a visit to us in Flint with his charming wife and three sons, all later to be distinguished in medicine. The family was on the way to Northern Michigan where apart from the cares and cares of teaching, of court duties and medical practice, he was accustomed to spend the summer months. Another choice recollection is of a reception at Oak Grove to Dr. Sawyer, president of the State Medical Society. Witty, versatile in story telling, he was at his best on this occasion and those who were privileged to remain late will not forget his contribution to the entertainment of the company, one of whom in sheer hysterical glee slipped from a chair to the floor.

Neither can I forget an afternoon's drive, to which he invited me, about Washington. Its history, its topography, its monuments were completely familiar to him. This was during the late war. What a fine soldier he was! How much the country is in his debt for meritorious service during this and the earlier embroilment of 1898.

In *A Doctor's Memories* (1926) he avers "My life has been determined by heredity and environment. These are the factors that have molded my being, given direction to its development, marked out the course of its growth and set bounds to its activities. Had either been different from what it was, better or worse, I would have been different from what I have been and from what I am."

In the same year I wrote as follows: "If one had his life to live over, it would be an exact replica of the past—his reactions to his environment would be identical. If environment or reaction differed in any particular it would not be 'his life'."

My last communication from him was dated at Washington, April 27, 1927, and reads:

"My Dear Old Friend

"I have just read your letter and your aphorisms. The former I greatly appreciate and the latter I endorse in toto. Although I am now in hospital I am hoping sometime in the near future to meet you in the flesh, when we will go over our common experiences."

"With love

"Yours truly,

"Victor C. Vaughan"

—C. B. Burr

Flint, Mich.

DR VAUGHAN AND POPULAR MEDICINE

DR VICTOR C VAUGHAN is remembered for his great services to medicine and the medical profession, with the same warmth of appreciation that surrounds the names of Pasteur, Koch, and other pioneers who were his contemporaries. In his years of endeavor at the University of Michigan, his work in combating the germ enemies within the gates of our armies in America's last two wars, in his inspiration of hundreds of medical research workers in his important part in the peace mobilization of science that followed the World War, he made contributions to civilization that would have brought fame to a dozen men less intellectually tall than he.

One major benefit he conferred upon the world, his own profession may not yet appreciate, so quietly and unostentatiously did he do his work. Dr. Vaughan was a pioneer among the advocates of the popularization of medicine. And like the best of advocates, he practiced his theories.

I have heard it said that when teaching his classes at Ann Arbor he found that if he used simple language instead of the more technical medical dialect, that may be heard in medical meetings even today, his students were better pleased and better instructed. In some cases the technical language of science is a convenient and necessary code for the concise expression of exact meanings, but in other cases it is a dialect that serves the same purposes as the secret language of Indian medicine men. Dr. Vaughan never hesitated to use plain English when it was most useful, he never failed to use the proper technical terms when they were necessary. His great works on epidemiology are delightful reading in spite of the fact that they are standard medical compilations.

From this insistence upon clarity in medical literature, it was a logical step to the phase of Dr. Vaughan's work that I knew best. The late Dr. Edwin E. Slosson and I were engaged in the beginnings of Science Service, an institution which has as its object (and I am glad to say our activities have borne fruit) the presentation of science to laymen in such a way that even those without technical training may understand. In our efforts to so express the facts of scientific research in vivid newspaper English that our stories would successfully compete with accounts of murders, baseball and politics, we were fortunate in having the encouragement and aid of Dr. Vaughan. He was chairman of the division of medical sciences of the National Research Council under whose roof we had our offices. We interrupted him when he was in the midst of writing portions of the volumes of his epidemiology; we listened to stories of his experiences in fighting disease, and I hope that others felt, as we certainly did, that our stories were the better for it. In the face of silent skepticism as to the possibility of telling the man in the street about science, Dr. Vaughan replenished our enthusiasms. More formally, he served as a trustee of Science Service.

I like to think that there was some slight reciprocal reaction that resulted from our drafts upon his knowledge and time. We discussed with him his ambition, soon to be splendidly fulfilled, to establish under the American Medical Association a reputable popular journal of medicine and health to carry medical and health information direct to the public. There were at that time financially successful so called "health" magazines that were doing much to spread medical misinformation.

Dr. Vaughan's success in converting the American medical profession to the radical procedure of publishing a lay journal of medicine is too well known to be told here. *Hygeia* is a monument to him.

So let there be added to other fond memories of Dr. Vaughan the fact that he was a pioneer in the popularization of science and an opponent of the idea that medicine is an art of which the public must be kept ignorant.

—Watson Davis,
Washington, D. C.

AN APPRECIATION

"A prince once said of a king struck down
"Taller he seems in death!"
And the word holds good, for now, as then,
It is after death that we measure men "

I MAY memorialize but could not measure a friend There is no rule to gauge the appraisals of friendship, to measure its boundaries or fix its limits

I waive any attempt to evaluate Dr Vaughan's contributions to scientific medicine No one could though others may list them Their influence in life-saving is beyond estimate or conjecture The direction of the first step, in any venture, is more important than its length The pioneer in scientific research must start from that known, which is so little, plant upon it that which he has discovered and protect their relation, before he may safely approach the unknown What seemed to us as intuition in Vaughan was conclusions reached from exact knowledge New vistas opened before him as he blazed trails thousands have since followed each erecting a marker, perchance a monument to scientific medicine, that the less patient or little skilled might read as they traveled in search of health for the sick or to protect the well against disease

The University of Michigan Medical School, for thirty years its Dean, is his imperishable monument Yet its students remember it largely because of its Dean

To have seen Dr Vaughan in his own home, presided over by a perfect American wife and mother, surrounded by five sons, mind meeting mind on the same plane of common interest, no restraint, no disrespect, all interested in that presented by either, the father with the boy's heart, the man's intellect, the scientist's knowledge, the guest feeling that he belonged there—that was the test of a man, matured in every essential attribute Example was his family monitor Precept was left to those who have nothing else to offer

Life looks upon death as a tragedy The universal, human passion to preserve this life, with that hunger for life after death, has created the many religions of mankind, all praying for future existence Even the agnostic's grave-side eulogy whispers the hope of immortality But he knew that the life of the little and of the great, alike, is rounded by sleep and, that man's future has been provided for First the life to be lived, then the sorrow of those left, which he dreaded but could not protect against Once he said, "I can truly say that with old age, so far as I have experienced it, I am content It is true that I am apprehensive—not of what may happen to me after death, but of what may happen before that event, or may happen to my loved ones before or after my departure In other words, only the things of this world concern me greatly " Of the death of his soldier son, in France, he wrote, " but we know that his fate awaits all, and that ultimately we shall join him either in eternal sleep or in whatever form of

conscious existence the wise Creator of the universe has provided for mortals, when their earthly duties are ended " In these lines is a measure of the man

My first relations with Professor Vaughan were those of pupil and teacher. Leaving the university I rarely saw him, not at all for many years but he was always to me, a presence. When in 1913 I asked if he would like to become President of the American Medical Association his characteristic reply was "Unsought, I would be highly honored. Sought, I would feel disgraced." He filled that position of distinction with unqualified credit to himself and to his profession.

The idol of his own household, the ideal of his pupils, the scientist, the unwavering friend—what more should be required of a man? To us "Taller he seems in death."

—Hubert Work,
Colorado Springs

THE VICTOR C VAUGHAN SOCIETY

ANN ARBOR, MICHIGAN

THE Victor C Vaughan Society is the crystallization of an idea to form an organization of those medical students interested not only in diagnosis and therapy but also in the history of the art, a field somewhat beside if not beyond the purely academic study of medicine. As happens in the organization of many societies, after being formed for diverse purposes, they perpetuate the names and ideals of men by taking the names of these men unto themselves. A group of senior medical students, having organized this society with a definite purpose and function, looked about for a name.

One cannot be associated with the Medical School of the University of Michigan for four years without being impressed with the singular significance of the name "Victor C Vaughan." His personality pervades the traditions of the school, making him revered by teacher and student alike. His deeds parallel the growth of this medical school and medical science itself during his regime. The nation knows the accomplishments that made him famous and has benefited thereby. It remains however for a few of us to appreciate the personality that did more for this medical school than all the accomplishments that were his. This organization then pays homage to Victor C Vaughan the man, and itself feels honored to bear such a name. In adopting it the society no doubt did more honor to itself than to Dr Vaughan. Thus also did the society take a long step in assuring its continuance.

Dr Vaughan never knew that this society carries his name as the letter at once asking his permission and informing him of the adoption of the name "Victor Vaughan Society" was in the mails at the time of his death.

The society as organized in the fall of 1929 consisted of twenty senior medical students and our faculty advisor Dr Samuel Altshuler. During the year, twenty of the most interesting men in medical history were discussed—each member having chosen to study and present the story of one man. Each paper presented was discussed by a faculty member who in many cases had been personally acquainted with the character presented.

The faculty responded splendidly and by their voluntary interest have been of untold help to us. Many opened their homes to us to hold meetings there, several faculty members made it possible for us to have lantern slides and photographs made and available for the meetings. All faculty members invited to participate in the discussions responded with an enthusiasm which was indeed stimulating to the organization in its infancy.

The names of the medical savants discussed and those reading the papers are as follows:

Vesalius by Vaughan V Morrissey
Thomas Brown by Wm Bromme
Sydenham by Richard Froyberg
Laennec by Samuel Drick
Roenigk, by Wm Coventry
McKenzie, by Spencer Braden
Pasteur by Charles Lemen
Lister by Park S Bradshaw
Hunter by Sherwood Russell
Furchow by Horace Boyden

Rush, by Robert Curry and Dr Adams
Bright by Elwood Mason
Beaumont by Chris Hindson
Schmeelckeus by Justin Neighbor
Oliver Wendell Holmes, by Philip Roche
Walter Reed by Frank Maxwell
Harvey by Harry Leivitt
Ehrlich by John Cameron
Picord by Frederick Lendrum
Osler by Donald LeDuc

The above papers are at the present time being prepared for publication in the *Michigan State Medical Journal*

The organization has recently elected twenty junior medical students who will carry on the work next year with Dr Thomas Findley as their faculty advisor. Thus do we hope to help perpetuate the name and ideals of Dr Victor C Vaughan

—Robert Curry,
 Ann Arbor

“A DOCTOR’S MEMORIES”*

NEGRO SLAVERY IN MISSOURI AS I SAW IT

NEGRO slavery was well established in Missouri long before it became a part of the United States. However, the number of negroes in that territory in proportion to the whites was never large. In 1860 the population of Randolph County, in which I lived, consisted of 8,777 whites and 2,619 negro slaves. In the whole of Missouri at that time the proportion of whites to negroes was as nine to one. The number of negroes decreased from the Missouri River to the Iowa border and in the uppermost tier of counties there were but few.

It is not my purpose to enter into any detailed description of the history of slavery in Missouri nor of the various legislative acts concerning the civil rights of the negro and his legal protection from cruelties that might be inflicted upon him by his master or others. I am telling only of what I saw of this institution on my father's farm and among our neighbors. The negroes in our community were divided into two quite distinct types. One had a black-yellow or tan skin. He was quick and alert in his movements, generally spare, muscular and graceful, above the average height of the other type, intelligent and ready in comprehension, generally good-natured and eager to join in every sport, though impulsive and quick to get angry. The males of this class were the artisans on the farm, carpenters, blacksmiths, tanners, harness makers, teamsters, etc. The females of the tan type were cooks, waitresses, house girls, spinners, weavers and dressmakers. The tan type of negro as I knew him was not due to admixture of race. He was not a mulatto. I am quite sure that the two types were distinct in their African homes, and, among us at least, they did not readily marry and intermarry. The tan type of negro, as I knew him, resembled the modern Zulu more than he did his comrades of the other type.

Those of the second type were coal black with thick lips and flattened noses, slow and somewhat awkward in movement, inclined to corpulency, highly superstitious and emotional in their religious conceptions, some of the males were great exhorters and some of the elder females great shouters in their religious revivals, inclined to great devotion to the whites and capable of making sacrifices for those whom they loved, most of them had great pride in the family which they served and resented the undertaking of any apparently menial service on the part of the whites, most of them were thoroughly trustworthy, slow but dependable and for the most part fairly industrious, greatly appreciative of words or acts of commendation, credulous and easily deceived, thoughtless and care free, willing to trust tomorrow and its needs to master, contented

*Editor's Note. Dr. Vaughan deleted the following three chapters from *A Doctor's Memories* in order to reduce its size.

While as they now appear they lack sequence we have incorporated them in the Memorial Number for the benefit of those who have read and enjoyed his autobiography and have expressed the hope that some time a second volume would be written.

to enjoy today and its blessings improvisors of meaningless melody with a soul full of music often struggling for expression, energetic but unskilled performers on the banjo or the violin

Louis, the most efficient of the tan negroes on our farm, made, with the help of his fellows and under father's direction, a family carriage, including all the iron and woodwork and the greater part of the harness. This vehicle would not have graced Connecticut Avenue though I have seen and occasionally still see more disreputable turnouts on that fashionable thoroughfare. The same artist made modest articles of furniture for the house and the cradles in which the babies were rocked and the coffins in which all ages and both colors were consigned to mother earth. Louis became quite a skilful carpenter and possibly he might without undue exaggeration have been called a fair cabinet maker. These and other encomiums I might pass upon Louis who, I will admit was one of my childhood heroes. However, his last act in my presence was one of the tragedies of that long ago time. Notwithstanding all the talk about freeing the negroes and their enlistment in the army, no negro on my father's farm showed any intention of leaving until the spring of 1864. One morning before breakfast I stood at the wood pile near the kitchen gate. Father and two or three negro men were near me. Louis, with an ax on his shoulder, was trying to drive a troop of young horses into the barnyard. One colt, in a spirit of playfulness turned and scampered by him. Louis viciously threw the ax at the animal. Father cried out "Louis what do you mean?" The negro picked up the ax and facing father said "You touch me and I will give you the ax" or words to that effect. Father made no reply but quietly turned and walked into the house. Shortly he was back with his rifle. Drawing a bead on the negro he ordered him to drop the ax. The man stood stricken with terror and slowly the ax fell from his hands. Father then told the other negro men to bind Louis to a post and to strip him to the waist. One of the big black negroes laid on the strap with gusto. The next morning Louis was gone and later we learned that he had enlisted in a negro regiment.

Within a few months after the above mentioned incident every negro on the farm and most of those in the community acceptable to the military service had enlisted in the army. In the winter of 1864-1865 when my father was hunted by the militia as I have told elsewhere the negroes aided in his hiding and on at least one occasion saved his life. Neither the military recourtements of the searchers their cajolery and promise of reward nor their threats could induce the negroes young or old, to reveal the whereabouts of their master.

When the family fled to Illinois in February, 1865, the house, the farm, and all that the marauders had left including some live stock, were left in charge of Uncle Jeff, who aided us in our departure and had as definite an idea of our intended destination as we could give him but to all inquirers, especially from those in military dress our disappearance was to him as unknown and unexplainable as if we had been taken to heaven in a chariot or swallowed into the earth. During the summer of 1865 Uncle Jeff, with such help as the negro women and children could render him, cultivated a few acres and reaped a meager harvest. On our return in the fall of that year everything was intact and turned over to Mars John in perfect fidelity. Uncle

Jeff and his dependents remained as tenants on the farm until death removed the elder ones and time dispersed the younger. Every third load of the harvest went to the negroes, while personal service and work in keeping up the place were paid for either in money or in produce. Surely no steward could have been more faithful to his trust than this old negro man. His care had saved the buildings and their contents from the despoliation and torch of the marauders who, during the late months of 1864 and the early part of 1865, rode over the fairest portions of my native state.

When the family in the fall of 1865 found itself again on the old Missouri farm, Mars John, with all other former slave owners in Missouri, found that his economic condition had been greatly enhanced by the emancipation of the slaves. In former times he had been compelled to house, clothe, and feed his negroes, through infancy, childhood, youth, manhood, and old age, in health and sickness, through nonproductive as well as productive seasons, whether the price of tobacco and hemp was low or high. Under the old régime if a negro, through ignorance, inadvertence, or viciousness did injury to a neighbor's property the owner was responsible. In slave days if a negro girl went astray, her mistress was, at least, criticized, probably ostracized by her former friends. The mistress of a house was expected to bring up her negroes in good behavior much as she did her own children. For a while the Missouri slave owner dreamed that he was being robbed, but on awakening and after rubbing his eyes and looking about he found that some one had lifted a heavy burden from his shoulders. Many of the slaveholders, certainly of those in Missouri, did not believe in the institution of slavery, but they did not know what could be done with their negroes should they liberate them. Now, the government had done this thing and had shouldered the responsibility. Possibly some of the then wise old heads, like Thomas Benton and others, had seen what should have been evident to all, that negro slavery in Missouri did not pay—that economically it was all wrong. Possibly some of them were mere sentimentalists as they were accused at the time of being. Sixty years have passed since the legislature in the state of Missouri freed its negroes, but the free negro problem has not been wholly and satisfactorily solved yet. The New England abolitionists of sixty years ago charged the slave owner with debauchery in concubinage of his more likely female slaves. Since emancipation the number of mulattoes has increased so rapidly that it is safe to predict that within a few centuries black negroes in the United States will be as rare as white elephants are now in Africa. Miscegenation, practiced but rarely by the slave owner, promises to be the ultimate solution of the negro problem. This is not a prediction for the future but a statement of a present fact. As to its effects upon both races I have decided opinions, but this is not the place in which to state them.

In the harvest of 1866, I, in my fifteenth year, driving a Wood mower, was cutting more hay in a day than seven lusty negro men with their scythes would have cut in seven days. Besides, driving a mower was a good thing for the boy. Had slavery continued, he would most probably have been doing something else, neither so pleasing nor so useful. Watching the heads of red clover fall did not always interrupt the boy's thoughts on what Caesar had to say in indirect discourse, nor, *mirabile dictu*, prevent his following the wanderings

of Aeneas is described by Virgil. One day, however, thoughts along these lines were abruptly and painfully interrupted as the flying shuttle cut through a hidden bumblebee's nest. The hitherto docile and lazy horses were instantly converted into their wild ancestors scampering over Russian steppes with their heels striking out madly for the four corners of the earth. The chariot driver, with equal celerity, threw out the gear and managed to check his flying steeds before they had reached the farther end of the eighty acre field, while his discourse became most positively direct. With this experience the boy learned a lesson not found in the writings of either Caesar or Virgil, but notwithstanding this he has not always been equally successful in the promptitude with which he throws his blades out of gear. In adult life the memory of the bumble bee incident has been awakened, when in anger I have not been able to throw my hot words out of gear soon enough to save me from humiliation if not from mutilation.

Negro dialect was discouraged among both whites and blacks in our community. I do not intend to say that our speech would, in all particulars at least, meet with the approval of grammarians. In fact, I am quite sure that had Missouri in or about 1850 been split off from the rest of the world and so continued up to the present time there would have developed among its people a distinct dialect. Dialect specialists assert that there is such a brogue, or at least there was a generation ago and that it was recognizable over the greater part of the territory west of the Mississippi since Missourians were the most numerous pioneers in this region. After I went to Michigan one of my close friends was Professor Hempl (late of Stanford University), an earnest and learned student of dialects in this country. With his dialect map of the United States he was wont to come to my laboratory where I was busy with microscope and test tube and ask me all kinds of foolish questions. He found it easy to place me in one of his larger groups which includes parts of Kentucky, Tennessee and Missouri because when asked to call 'cows' I said 'sukev' and not 'co bos'. I carried water in a bucket and not in a pail. I designated a frequently used implement in cookery as a skillet and not as a spider. I bought butter by the firkin and not by the ju or tub. I wore galluses and not suspenders. Hempl said that the only Missourianism I employed was an affirmative grunt something like 'uhum'.

Among my grandmother Dameron's negroes was a couple who in my boyhood days were very old. To the children they were 'Daddy' and 'Mammy'. To the other members of the family they were Uncle Harry and Aunt Esther. 'Daddy' was no Beau Brummell. His face the small part of it which one could see was coal black. His features were mostly hidden by his abundant woolly hair and whiskers as snowy white as the fleece of the whitest sheep and I must add that they were never stained by tobacco. His underlip turned down and quite constantly the saliva drooled from his mouth keeping him busy wiping it away which he did with the back of his hand and his shirt sleeve. The backs of his hands were as black as pitch and his fingers were long and loose jointed with no signs of arthritis. He had no tasks at least during the time I knew him but was far from indolent. One generally saw him with an ax on his shoulder or cutting weeds and bushes in the fence corners with a

brush hook. He had frequent visits and conversations with the devil whom he regarded as by no means as evil as his Satanic majesty is held in our estimation. His devil rode in a small coach drawn by tiny black horses, sometimes by black dogs. Occasionally he rode some black animal, horse, dog or cat. His Satanic majesty gave Uncle Harry exclusive and certain information concerning both past and coming events. He was especially strong and reliable in foretelling the future, generally pertaining to the weather or to the crops in different fields. In short, Uncle Harry was a senile dement with harmless hallucinations and delusions. To the children he was most gentle and kind. He believed that he had been brought from Africa, but of course this could not be true. Grandmother said that he had been in the family beyond her memory, but in her childhood he had been an efficient man. Aunt Esther was younger and less infirm. She did not believe in Uncle Harry's devil but she was full of stories of "raw heads and bloody bones," which she poured into my ears as I sat on her lap before the great kitchen fire in the winter or before her door in the summer. For a while Aunt Esther's stories so frightened me that after listening to them I was afraid on a dark night to walk alone the few paces from her cabin to the back door of the house, but, being assured by mother that they were mere stories, I teased for them as a child does for fairy tales. However, I did once see Aunt Esther's "raw head and bloody bones." I had grown old enough to help do the chores. One evening while it was still light, accompanied by two negro boys, I was sent to the woodland pasture to find and drive in some horses. We lingered under the mulberry tree until it was growing quite dark. Then, with some anxiety, we began our search for the horses. They had strayed farther than usual and when found they were not inclined to follow our directions. In this part of the pasture there was a neglected graveyard unfenced. I was heated and angry. While running after the horses my foot struck a grave stone and I fell sprawling full length on the mound. As my foot struck the stone I was uttering a swear word and before I could rise I saw the apparition which Aunt Esther had so often and so graphically pictured. I told mother about it and she said that my vision was pictured by my conscience and was due to the swear word.

Most of our negroes were deeply religious. While the whites were divided into many sects the church of preference for the negroes was the Baptist, though many affiliated with the churches of their own white folks. There were in our community at that time no separate negro churches. Negro preachers were numerous and exclusive services were occasionally permitted in the churches for the whites. Negro revivals were most frequently conducted in some grove with one or more whites present to see that the exercises were kept within bounds. For the most part, however, negroes attended church with the whites, a railing or a gallery setting apart the seats reserved for them. Basket dinners were common in warm weather. The whole family, white and black, with hampers of food, in farm wagons, drove to the church. After the morning service a picnic dinner came with visiting, gossiping, crop discussions, and more or less love making among the young people of both colors. Then there came a second sermon, a christening, a baptism, or some other function. After emancipation quite a discussion arose as to the propriety of the whites at-

tending serviees with the negroes. The congregation was divided and some un-Christian things were said. In the midst of this feeling it was decided to awaken a revival. An elderly preacher, with some local reputation in bringing sinners to repentance, was chosen to give the first sermon. He had spoken vehemently, if not eloquently, for an hour or more when he began a peroration, something as follows: "Brothers and sisters, saints and sinners, old and young. The good old ship Zion lies at the wharf. Who will go aboard? Her sails are filling with propitious winds. Who will go aboard? She is bound for the heavenly shore. Who will go aboard? After multiplying these descriptions and these questions the good man stood disappointed at his failure to secure response. At this juncture a huge black mammy on a brick seat arose and started down the hall. The preacher was dismayed. On the front seats he saw those who had vowed that they would not worship with freed negroes. He hesitated and then his Christian spirit prevailing he stretched forth his hands advancing to the penitent and cried out: 'Come on mammy, come on! I will take you on board if it sinks the old ship.'

The health of the negro slave was looked after as well as the knowledge of the time permitted. In the fifties a strong vigorous negro man in Missouri was worth or at least sold for \$1 000 to \$1 500; sick he was worth nothing. For selfish if for no other reason, the owner did not imperil the health of his property. Even marks left by whipping diminished the negroes' value not so much on account of the physical injury but as an evidence of the vicious character of the bearer. The same doctor administered to whites and blacks and charged the same fee for each call. There were in our community at that time no chiropractors, no osteopaths or other cults. All practitioners of medicine were graduates mostly either of Philadelphia or Louisville schools. From a hygiene standpoint there was undoubtedly overcrowding in the cabins but most of these were built of logs and with a huge fireplace filled with burning wood there was no need of artificial ventilation. The food was the same as the whites had with the exception of certain delicacies like squabs. There was plenty of it and vitamins were not wanting in the diets of those days. I once heard mother chide Louis for leaving so much on his plate. Lord Miss Adeline, how does I know that I get enough unless there is something left? The clothing, possibly with the exception of Sunday suits, was all made from fibers grown on the farm and there carried through every step in their manufacture. Whites and blacks wore in summer coarse linen and in winter rough jeans.

The two diseases, tuberculosis and syphilis, now so prevalent among the negroes were rare among Missouri slaves. In some communities tetanus of the newly born was common. This was due to lack of asepsis in dressing the cord. This fact was ascertained by the country doctor in the South long before the discoveries of Pasteur and Lister. In the preface to my work on *Epidemiology and Public Health* I have said something about the sanitary conditions of the Missouri farm in my boyhood days. As we now see it, it was pitiful. The average life was short and the death rate was high, but this was common throughout the country and it applied to both races alike.

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nog in front of her. Each member of the family, first the whites and then the blacks in order of age received from her a glass of this delicious beverage and drank to her health. Christmas was then supposed to be inaugurated and the holidays were to continue for one week and as much longer as a trace of the backlog could be identified. If my memory does not betray me, the gam was usually from one to three days. Of course, everyone will understand that a vacation on the farm cannot be absolute. Fires must be kept going, food must be cooked and served and stock must be watered and fed, but all nonessential work ceased during the holidays.

There was no Christmas tree but much giving of Christmas gifts. In this there was a certain etiquette. The one who cried out the name of some other member of the family and then added 'Christmas gift' was entitled to some thing. Yet between whites and blacks of the same ages it was never proper for the white to call out first. White children might claim gifts from adult negroes and I may add always had them, especially from 'Daddy' and 'Mammy,' but with this exception it was always the proper thing not to see the servant on Christmas morning until he cried out 'Christmas gift' and then one should seem much surprised. The children of the house had great fun watching the servants on Christmas morning pretending to hide behind the leafless shrubs in the yard in order to catch a Christmas gift from the master of the house who was always stone blind on that morning, and the look of pretended surprise on his face when he raised his eyes in recognition of the greeting was a joy to all. 'Oh, Mars John I caught you that time' was the gleeful cry that came from the mock hiding place. Then the master's eye would fall, after acknowledging that he had been taken wholly unawares to be equally surprised at the next call. The mistress went through a like ceremony with the house servants and when all had assembled in the dining room before the buskily burning fire with the great water soaked backlog the gifts so recently claimed but long prepared in anticipation, were piled on the table ready for distribution accompanied by kindly words in recognition of services faithfully and cheerfully rendered. The scene certainly was in strong contrast to the overdrawn pictures of *Uncle Tom's Cabin*, exceptional in reality in Missouri.

One of the pleasing mysteries of the farm was the location of the private watermelon patches. In addition to the large patch for the whites each adult servant had his private patch and the location of this was supposed to be known only to the individual owner. The watermelon seeds were always planted on the first of May before breakfast, and after the large patch had been seeded, each adult servant took himself and his well selected seed to the corner of the farm where he had already prepared the ground. All through the summer and fall the locations of the patches of Uncle Dick, Uncle Jeff, etc., were supposed to be known only to their respective owners although there was not a nook of the farm unvisited by the barefooted sons of the farmers and their daily companions of color. Melons always grew to the largest size and had the reddest, sweetest hearts in Uncle Dick's patch and it was with much pride that he occasionally brought from that unknown spot one of its largest products as a gift to the family. The barefooted son of the house had often looked with covetous eyes on the great green striped melon that grew only under the

magic cure of Uncle Dick's hoe, and once the temptation proved more than mortal boy could bear, but, after the interview with his father the next day, he learned that although stolen fruits may be sweet to the palate they are prone to sour in the stomach

Once or oftener each summer there was a barbecue, usually in the latter part of August, after the corn had been laid by and the tobacco hoed, primed, topped, and succored. Pits were dug, filled with logs and brush and great fires roared. Pigs, sheep, and oxen were dressed and suspended over the glowing coals. Much of the preparation and the cooking were done during the day and night preceding the assembly. Often the barbecue was the occasion for some political event. Candidates from governor down to constable were present, and in a large auditor much eloquence was poured out upon both attentive and deaf ears. The negroes were there en masse, some as cooks and waitresses and some as lucksteers. Many of the latter had booths arrayed and arranged most diversely. On the rude shelves were great green watermelons, yellow cantaloupes, cakes and lemonade, all for sale. Little white boys vied with one another in pointing out the superiority of the displays made by their family negroes. I was a hawker for "Uncle Dick," and I never failed to receive my commission. In the afternoon when my allowance for the day had been exhausted and I came at the head of a small troop of white boys loudly proclaiming the superiority of Uncle Dick's wares, he, or more likely Aunt Mary, his wife, would call to me. "Victor, here's a big melon what has just busted itself. You boys go and eat it."

While on such occasions I frequently saw a drunken white man, I never saw a negro in this condition. Possibly fear of punishment restrained the latter, but so far as my observation went, drunkenness was not a common vice among the Missouri negro slaves. A wider observation in later life leads me to conclude that next to the red man the white man is the most ready victim to alcohol. Furthermore, I believe the more freely the blood of white and black have mingled, the more generally has the latter developed the vices of the former. Be this as it may, I never, during slave days, saw a drunken negro on my father's farm, although there was always in the house, and not under lock and key, a jug or a demijohn of whiskey. While on this subject I may add that one of the earliest of my recollections was being sent to the garden every morning in summer for the mint for my grandmother's mint julep, and as a reward I was permitted to eat the excess of sugar in the bottom of the glass. According to certain teachings, I should have grown up to be a drunkard. While I never was in pre-Volstead days a total abstainer, alcohol has never been a temptation or a danger to me.

We had one negro, George, who stuttered painfully and swore volubly. I have always thought that it is no sin for a stuttering man to swear. Certainly it does loosen his tongue. I have an idea that even my mother looked upon George's profanity as an infirmity rather than as a sin. One morning I learned that George had "got religion" the night before at a revival then in progress. As we hoed tobacco side by side, I said, "George, I suppose you will swear no more." He leaned on his hoe handle, his lips twitched, his whole facial structure went through violent contortions. He made a desperate effort, but

he could not begin without a "damn." And George went through life with many 'damns.' I hope that the recording angel did not keep tally. In George's speech an oath was what the starter is to a gasoline engine.

The white children were taught to be respectful to the blacks. If I failed to thank the waitress who passed the hot biscuits, I was not allowed another at that meal. I acquired so deeply the habit of thanking the waiter that I still do so in hotels and restaurants and I have often been embarrassed by finding myself quite audibly thanking the waiter at a private dinner. The small white boys were also taught to tip their hats to the elderly negroes. Parson Root, of whom I speak elsewhere, was once criticized for tipping his hat to an old negro. What, 'said the parson, 'should I show myself less of a gentleman than the old negro?' On my visits to Missouri in recent years I have never seen a white boy tip his hat to an aged negro. So far as I remember, the only time my father ever laid his hand on me in anger was when I changed a request of his into a command. Preparing to shave, he told me to ask a negro girl to bring him hot water. I went to the door and shouted, 'Lucinda, father wants hot water for shaving step quick.' My command had hardly reached its recipient when a heavy hand fell upon my shoulder and sent a painful impression through every part of my anatomy as father asked, 'Who told you so to say step quick?' I sinned no more, at least not in that direction, in the presence of my father.

In the fall we frequently indulged in hunting coons and opossums down in the creek bottom. We frequently found the opossums hanging by their tails from pawpaw bushes. The coon was captured with more difficulty and it was quite an event in life to bring home one of these ring-tailed animals. Proud indeed was the boy who could wear a coonskin cap with a tail hanging down behind. This honor was supposedly reserved for those who had actually participated in the capture of the animal. Indeed in our neighborhood for a boy to appear at school under a coonskin cap was a distinction. It was a badge of honor, a medal of bravery, and an announcement to the world that the wearer was worthy of the honors of manhood. From my eighth until my twelfth year I frequently accompanied the negroes on their coon hunts. There was no need of starting early, since it was supposed that the wily coon did not leave his hiding place until the shades of night were well drawn. With a half dozen or more hounds we would go into the great forest. Soon the dogs were busy and when one of them let out a cry, negroes and small boys followed as rapidly as brush and swamp permitted. On successful nights which were rare the dogs tired the coon. Generally the animal was wise enough to select a large tree. Under this a great fire was built and occasionally it happened that Mr. Raccoon grinned down upon us from some inaccessible branch. I never knew a negro to show evidence of laziness in a coon hunt. However great the tree and however dense its wood there were always volunteers to play the ax. The critical moment came when the tree fell. The dogs were immediately among its branches and the hunted animal either escaped or made fight. Choosing the latter alternative always met with disaster. Dogs and negroes were too much for the coon once within their reach. One coon a night was sufficient to satisfy the most ambitious hunter. When success

crowned our efforts we had a great war dance about the fire. The negroes sang weird chants and dirges. Some of these still run through my memory chambers, but I would not dare to try to reproduce them. On many a coon hunt I was the only white among a dozen or more negroes. Some may say that my parents were neglectful of my training and that I had opportunity to enlarge my vocabulary of vulgarity. I can truthfully say that I never heard a negro slave tell a dirty story. My morals were in no particular corrupted by my associates. Many a time while hunting coons some negro man has carried me on his back over rough or swampy places when I had grown too tired to keep up with the desired pace.

I am sorry to say that the negroes, in my neighborhood at least, did not continue their reputation for industry and integrity after freedom came to them. On my second summer vacation at Michigan I went back to the old farm. On the morning after my arrival I wandered to a distant wheat field where four negro men were supposed to be busy cradling the grain. I found them playing craps in the shade near a small stream. I stripped myself of my outer clothing and told them I would shock the bundles as fast as they cut and tied the stalks. These negroes did at least one day of full work, but the next morning I did not get up. The doctor pronounced it a case of typhoid fever, but I have always thought that it would have been more correctly diagnosed as fatigue fever. At least it continued for some two weeks and I did not repeat the foolish experiment.

RUSSIA IN 1897

THE International Medical Congress met in Moscow in 1897, and I had the pleasure of being a delegate and taking a part in the proceedings. In this chapter I will not discuss the learned papers and epoch-making discoveries presented but will tell of the incidental observations and experiences some of which subsequent events have brought into prominence. Our family decided to make the trip an occasion for the extension of our knowledge of the big world and those who dwelled within the regions to be visited. Early in June, Mrs. Vaughan, the three older boys and I, with our bicycles, landed at Antwerp. A few days later, about mid afternoon, we rode into Brussels in a heavy rain, with our scant belongings attached to our handlebars. We had our membership cards in the Touring Club of France and our guidebook directed us to the Hotel Bordeaux. We found this hostelry uninviting and the proprietor seeing our predicament showed us undesirable apartments* for which he demanded unreasonable prices.

I had been in Brussels before and knew something of the city, so I said that we would go to the Hotel de l'Univers. This provoked the mirth of the boniface who predicted that in our dress we would not get farther than the door. This decided the matter and within a few minutes we were telling our story and making our appeal for shelter to the dignified, beribboned porter of the more inviting hotel. Soon we were drying our garments before a big fire in a spacious, handsomely furnished room. When

the dinner hour approached, the porter came, asking if we desired our meals served in our rooms or would we go to table d'hôte in the large dining room. Asked about the fitness of our raiment, he told us that an Ecumenical Council of the English church was in progress in the city, that some of the chief dignitaries were residing in the hotel that these might regard us inquiringly but that other guests would look upon us as a distinguished bishop, possibly an archbishop and his family so to the dining room we went. We gave some days to seeing the capital of Belgium during which time our gastronomical needs were supplied at the bountifully laden table d'hôte. Some months later, Mrs. Vaughan, one of the boys and I, properly clothed, drove to the Hotel de l'Univers. As the dignified porter greeted us he asked 'Where are the other boys?'

Leisurely and pleasantly we rode through Waterloo, spending a day on the battlefield, Mons, Maubeuge, Guise, Le Fer, St. Quentin, Soissons, Compiègne to Paris. It was cherry time and we made the purchase of these and the drinking of light beverages excuses to stop, linger in the homes of the peasants and try out our French. Previous visits had made us acquainted with Paris, its beauties in architecture and city decorations, its treasures in art, literature, and science and its wickedness but this was our introduction to the rural and village population of France. These were joyous days spent in touring this portion of la belle France. Little did we dream that the clouds of war were to anchor here for more than four years and pour down their missiles of destruction on this part of the earth that its productive fields, then rich with harvests of fruit and grain would be plowed by shells of dynamite that its small cities and villages then overflowing with hospitality and comfort to us as wayfarers would be sacked and burned by savage Huns that even the air which we then breathed so joyously would be filled with poisonous gases that the fair prospect upon which we then looked would be converted into an inferno, surpassing in its terror and cruelty the descriptions of hell by Dante, that our sons would be called to aid in arresting the ruthless progress of the invader that our firstborn then the leader the life and the joy of our party, would today be sleeping in the soil of France.

Some are now inclined to chide France for her continued resentment toward Germany. It is easy to forget injuries done another but he who has felt the heel of the barbarian on his neck long bears the scar.

Before leaving Ann Arbor, I had received from the secretary of the Moscow Congress a letter asking me for the names of our party and of the points where we intended to enter and leave Russia and suggesting that I call upon my Paris banker for further information. On doing this I found passports into Russia relieving us from all customs inspections and granting free transportation for all members of the party over Russian railroads.

A few weeks later the small hotel at Vevey where we were stopping was thrown into excitement by the arrival of a gentleman from Russia with his family, numbering fourteen. The next morning as we sat in the spacious garden, looking out over Lake Geneva the Russian gentleman joined us saying that we were Americans and he wanted to know us. He was Mr. Alexander Barry, born and reared in St. Petersburg, graduated at the Polytechnic School

at Zurich in 1872, went to America, worked for two years as a day laborer in the car shops of Jackson and Wiley at Detroit, met with an accident resulting in a broken leg, was under the care of Dr Herman Kiefer, returned to Russia, had shops in seven cities, employing in each from 2,000 to 3,000 men, resided in Moscow, was returning to that city in a few days, would secure hotel accommodations for us and would meet us at the station on our arrival

We traveled via Munich, Vienna, Cracow and Warsaw, lingering for a few days at each of these places. We had left our two older sons at Vevey, and were joined at Munich by Dr Alice Brown and at Warsaw by Dr Novy, his wife and two sons, so we made up a party of eight

I had promised Mr Barry to telegraph him on leaving Warsaw. The hour of the train's departure was uncertain, as was then the case with all Russian trains, so I did not telegraph until the train had started. I was informed that the telegram could be sent only in Russian. After some search through the train I found a Warsaw doctor who kindly made the translation and relieved me from further care in that matter. We left Warsaw about 10 A.M. and reached Moscow about 5 P.M. the next day. The train was a special of first-class cars with sleeping accommodations and filled with delegates, among whom we found many friends, American, English, French, and German. At Minsk and Smolensk we were greeted by reception committees and were provided with abundant refreshments. We found Mr Barry in his rubber tired carriage drawn by Oloff horses at the Moscow station and soon were in comfortable rooms at the Hotel Shveitsarna. We had asked Mr Barry to secure rooms for us in a distinctively Russian hotel, we did not wish to be in a cosmopolitan hostelry. We were the only foreigners in this house and none of its staff spoke any foreign language.

Having deposited us with our luggage at the hotel, Mr Barry drove away, saying that he would call in half an hour and take us to dinner. We drove to Petrovsk Park and dined at the famous Hermitage Restaurant. It was a distinctive Russian dinner, everything, except tea and coffee, being native products. We selected our fish as it swam in the pool. The wines including a fair champagne were made from grapes grown in Russian soil. Mr Barry's hospitality was unbounded. His family being in Switzerland, he called his sister from her summer home, opened his house, and insisted on our being at home.

The day after our arrival proved to be the holiest of the many holy days that then bedecked the Russian calendar and Mr Barry came early and took us through the Kremlin. As a guide he was so efficient that soon, as many as could keep within hearing distance joined our party, and I was surprised at many of the statements he made. Standing by the great bell he said "We Russians say we have the greatest bell in the world, but it was never rung, the greatest gun but it was never fired, the greatest Czar but he never rules, the present Czar is an untamed boy and we must wait some years before we can place an estimate upon him." I was greatly surprised at the freedom with which not only Mr Barry but his friends, to whom he introduced us, spoke of their government, its institutions and their management. Should I today in Washington speak as critically of President Coolidge and his administration

as the Russians in 1897 did of then Czar and his ministers, I should expect to see the cold shoulder of many of my friends turned toward me, if I met with no greater evidence of disapproval.

At a dinner I quoted a French author who said 'Russia is an absolute democracy in an absolute monarchy.' 'Yes,' spoke up Mr. Barry 'That is the exact truth and if there is anything worse than an absolute monarchy it is an absolute democracy.' Then he proceeded to tell how franchises were secured from mires by bribing the elders, how titles were bought and sold, how the church impoverished the people by feeding them upon superstition and exacting tithes, how the governors of the provinces bartered privileges, etc. etc. I received the impression from these educated Russians whom I met, the number of course being small that they were not blind to the many and obvious defects of their government. I could not discern that they had any religion. The uneducated and I was told that illiteracy then included more than 70 per cent of the population of the empire, regarded their government as a politico religious burden placed upon them by heaven and were at heart bitterly antagonistic to their priests who so far as I could ascertain, were for the most part an ignorant idle pestiferous lot. I am not at all surprised at the antic church attitude taken by the Soviet Government.

From the Kremlin Mr. Barry took us to a Metropolitan service at the Church of Our Saviour a splendid marble temple built by the Russians as a thanksgiving to God for their deliverance from Napoleon. The great cathedral was packed with thousands of standing natives interspersed here and there with small groups of foreigners. The service was in old Russian which nobody, with the possible exception of the Metropolitan understood. There was a choir of 400 male voices without any instrumental accompaniment. Such church music I have never heard elsewhere. The sound waves began in the distance, approached nearer and nearer and swept through and over the great throng in overwhelming harmony. At one time I found myself quite isolated from my party and wedged into a group of ragged dirty pilgrims. Their faces were ecstatic with religious fervor. One tried to prostrate himself and bring his forehead to the floor, but the crowd was too dense. I saw another with every visible evidence of extreme poverty hunt through his rags and draw out a copper which he bestowed upon a companion apparently even more pinched by want than himself.

One of the happenings at the Congress has gotten into current literature in distorted form. The city of Moscow voted a prize of 10,000 francs to the living man who had done the most for medicine and asked the Congress to name the recipient. The Congress did the only thing it could do, it appointed a committee to select the man. The chairman was the great German pathologist Rudolf Virchow, and fortunately I was one of the members. Here was a chance for a reenactment of the story of the apple of discord. The committee was in a quandary and greatly regretted the duty imposed upon it. For obvious reasons the recipient could not be a man from one of the great nations. There were two or three meetings. The wise chairman who was a politician as well as a scientist said 'We will prayerfully consider this matter, meet Sunday morning and decide it.' We met in the gorgeous hall of the nobles, the chair

man, tactfully speaking in French, proposed the name of Henri Dunant, the founder of the Red Cross, giving as his qualifications his personal poverty and his nationality, a Swiss. After Dunant's death I read in several European and American publications the statement that the poverty of his later years had been relieved by a pension granted him by the Empress of Russia. At the next meeting of the Congress the Moscow pension, which was to be continuous, was awarded to a Spaniard. I do not know what has become of it since. Alas! International Medical Congresses are, temporarily at least, moribund, and Moscow has probably had other uses for its money. These International Congresses of Medicine and other sciences seemed for a while to promise peace between nations, since science saw a multitude of ways in which the race might spend its energies more profitably than in war. Had the Kaiser had the vision and intellect of Vnehow the great catastrophe might have been averted.

We had great fun with the dioshky drivers. There were no fixed tariffs and the rule was that one must bargain with the driver before entering the cab or pay whatever he charged on alighting. We would wander to distant parts and then stop at some corner and call "Hotel Shveysarna." Soon there would be about us a host of drivers and the bargaining would begin in pantomime. They would indicate by the sweep of the arms a great distance and by the fingers the number of rubles, generally beginning with ten. The price would have been more had the men possessed more fingers. We would make our offer by extending fingers, generally beginning with one. The play was invariably interrupted by some native addressing us in German, French, or English. "Brother, you are a stranger, where do you want to go? How much does the driver want? Get into this cab, the charge will be so and so."

One evening I beat a driver down to a ridiculous sum. He was to take us to Petrovsk Park, wait until we dined, and drive us back to the hotel. On our return I handed him three times the amount agreed upon. He began to make the change, I made him understand that he was to keep the whole. Dull as he was he was not slow in comprehending me on this point. He dropped the reins, threw his arms about me, and, drawing me to his breast, kissed me on both cheeks. I never tipped another dioshky driver.

The Polish Jew in Cracow on receiving a gratuity lifted the donor's coat tail with ceremony and kissed the hem of the garment.

The Grand Duke Sergius, afterward assassinated, received the delegates and there were many banquets and musical entertainments. We heard much good music in Russia, then the breeding place for great musicians. We heard Chaliapin for the first time and since his coming to this country we have never lost an opportunity. In my seventy-fifth year I would walk a mile tonight and pay the fee from my scanty purse to hear him repeat *The Two Grenadiers*, then every nerve in my anatomy thrills from its roots in brain or cord to the minutest ramifications. In my opinion, the Russians in both music and literature are the most natural and realistic people in all the world.

We spent some hours in a convict camp where about three thousand had been assembled preparatory to transportation to Siberia. There were no political prisoners among these. When a community (mir) decided through

its board of elders that a member was unfit to continue in it, he was not sent to prison or hung, but his name was stricken from the roll, he was declared legally dead, and was sent to Siberia. If the condemned man had a wife she could accompany him or remain as a widow. I cannot think that cruelty is a Russian characteristic. He may permit suffering through his ignorance, indifference or fatalistic attitude of mind, but the practice of my barbarity I believe to be foreign to his nature. Possibly a wider acquaintance might change this opinion.

Nothing could better illustrate the utter ineptness of the Russians in practical matters than the way they failed to provide for the transportation of their guests at the International Congress of 1897. Even the Soviet Government has made great improvement in this particular. Their hospitality was prodigal in an extreme degree, but it was unorganized and resulted in much confusion and discomfort. Each delegate and each member of his party carried a pass from the point of entering to that of leaving the country. The special trains consisted of compartment cars, each compartment providing for four passengers and convertible into a most comfortable bedroom at night, but there were no reservations and one had to risk limb and life in securing a place. We experienced some discomfort on our ride from Warsaw to Moscow, but later we saw an unbelievable tumult. Some hundreds of delegates were to go one night from Moscow to St. Petersburg we among the number. For two days before notices in many languages were distributed and posted, saying that at 7 P.M. the regular train carrying no delegates would leave at 7:30 and again at 8 o'clock special trains for delegates only would go. Each delegate was asked to have his pass stamped before entering a train. Mr. Barry promising us that we would see something we had never seen before, took us to the station before 7 o'clock seated us in a gallery where we could look down into the spacious waiting room, told us to keep out of the crowd not to worry, and assured us that we would ride to St. Petersburg that night in comfort. There we sat comfortably smoking, drinking tea, and eating cakes. An old man, who might have sat for a portrait of Moses, or some other Hebrew patriarch stood at a small table in the waiting room with his official stamp in his hand. Delegates in droves of fifty or more shouting and gesticulating in all the languages of the civilized world filled the waiting room crowded about the old man, knocked over his table, and disregarding the stamp filled successively each of the three scheduled trains. Two or three policemen did appear but they were treated with the same rude courtesy bestowed upon Moses. When the last train pulled out an announcement was made that there would be no more trains for St. Petersburg that night, and the lights were turned down. Mr. Barry put us into his waiting carriage, told the coachman to drive about the parks and to return by 11 o'clock. There was a beautiful moon and we enjoyed the ride and tried to recall scenes from Tolstoy, Turgenev and Dostoevsky. On our return we found the waiting room lighted and occupied by a moderate number of quiet people among whom we did not see a delegate's badge. Each of us had a comfortable bed and passed over the 400 miles of smooth road in dreamless sleep.

When the door of our compartment opened in St Petersburg the next morning, we were greeted by a handsome man in the full dress uniform of a colonel. This was the foreman of Mr Barry's St Petersburg shops, who had secured rooms for us on Nevski Prospekt, and under whose guidance we saw the city built by Peter the Great and its environs. The institution which most interested us was that for experimental medicine, situated on a beautiful wooded island, encircled by the branching Neva and occupied by the most eminent of Russian scientists who with every available facility were devoting their lives to research. Medical men know of the great achievements secured by their labors. Here Dr Novy and I met Fraulein Schultze, who had been a fellow student with us in Koch's laboratory in 1888.

Before we left Moscow Mr Barry told us that he would be in St Petersburg on a certain day, would again assume the rôle of host and guide and had in mind a wonderful treat for us. By that time we had come to believe that all things, in Russia at least, were possible to Mr Barry. When he told us this, he wore the countenance of a small boy who had secured a present and could scarcely withhold the desire to inform the recipient of its great value. Several times he repeated "Be up early and have your breakfast before I come." We knew from the daily papers that President Faure of France was then visiting the Czar, but we never suspected that this event had any connection with Mr Barry's treat. He did come early and impatiently tore us away from a half-finished breakfast. In droshkys we were whirled down Nevski Prospekt, across the bridge ornamented with the wonderful horses, and soon were at the quay, were rushed into a waiting boat and quickly carried to Cronstadt. Here we were transferred to a larger boat and on looking about saw three French men-of-war. Suddenly the band on the French flagship began the melodious and plaintive strains of the Russian national anthem and the Czar's yacht bearing its imperial owner and the French president came out from Peterhof and anchored alongside the flagship. Some hours were devoted to the parting formalities. Many decorations were bestowed, toasts were drunk and lunches served. While we looked on, we were introduced to Mr Barry's friends on our boat and had opportunity to finish our breakfast. Among the many interesting men we met the most charming and communicative was the colonel of the Cossack Regiment constituting the Czar's bodyguard. This man had evidently learned his English by reading an English translation of the Bible. He talked in biblical terms and the burden of his communications was the injustice with which other European nations had treated Russia. As I remember, his discourse ran as follows: "In 1877 we buckled on the armor of Christianity and went down and smote the Turk hip and thigh, then Bismarck and Disraeli robbed us of all we had won. Had it not been for these men there would now be no Armenian atrocities, there would be no Turkey in Europe, but I shall live to see the day when the Russian service will be heard in St Sofia, the Triple Alliance is against Russia, now France is with us, France and Russia can withstand the Triple Alliance, but the question is what will England do?" I hope that this man lived long enough to see his question about England answered as it was in 1914, but that he died before the Russian debacle came in 1918.

Some time after noon the French fleet took its departure, the Russian ship leading the way, the French flagship with President Faure on the bridge following, escorted on each side by a Russian ship on one of which we were. The French bands played the National Anthem and the Russian band took up the Marseillaise, while cannons boomed from the shore. In the midst of this excitement I found that my son had drawn from his capacious pockets a small American flag and was waving it most lustily. I cautioned him but the Cossack colonel, lifting him on his shoulders, told him to wave it. So far as I know, this was the only American flag displayed in the Gulf of Finland that day. In 1912 I was telling Professor Vladimiroff of the day in 1897, when he said "You heard the Marseillaise the first time it was officially played in Russia since Napoleon's invasion."

Late that evening we returned to St. Petersburg tired out with the exertions and excitements of the day, wholly unconscious of the fact that we had witnessed a great historical event and with no vision of the world disturbance with which it was to be connected.

We returned to Paris via Helsingfors, Abo, Stockholm, Lübeck, Hamburg and Berlin but met with no unusual experiences. Our Russian journey widened our knowledge of the world and of the people living therein enabled us to follow more intelligently the catastrophic events that followed and stored our memories with reflections upon which we are dwelling in our old age. We kept in occasional touch with Mr. Barry until the war since we have been unable to ascertain anything about him. We would welcome an opportunity to return some of the many kindnesses he bestowed upon us.

THE MICHIGAN STATE BOARD OF HEALTH

I SERVED on the Michigan State Board of Health from 1883 to 1919 with an interval of two years. In the State I have always been known as a Democrat although as I have elsewhere indicated my politics both State and national has fluctuated from time to time. Any one acquainted with the political complexion of Michigan will understand that my appointment on this Board must have been largely in the hands of Republican governors. I have heard those interested in public health work urged to steer clear of party affiliation. I can say that the leading Republicans in Michigan have for the most part, been above petty politics in all matters pertaining to the health and welfare of the people. My first appointment on this Board was made by a Democrat, Governor Begole. Much good natured chaff was thrown at this man on the ground that he was not educated. Be this as it may, he was not devoid of either wisdom or wit. In addressing the American Public Health Association at an annual meeting in Detroit he spoke of physicians coming together for the purpose of limiting the spread of disease and said "If I should be called upon to address a gathering of lawyers assembled for the purpose of preventing litigation, I would say with Simeon of old 'Lord, let thy servant depart in peace for mine eyes hath seen thy salvation'."

At the expiration of my first term, the Republican candidate for Governor, Mr Luce, was a warm personal friend and coworker in health matters. I went to the polls intending to vote for him but on my appearance at the booth a group of Republicans shouted out that I must vote the Republican ticket or they would see that I would not be reappointed. Angered by their threat I picked up a straight Democratic ticket, the only unscratched ticket I ever voted and deposited it in the box. At the expiration of my term Governor Luce reappointed me and with my commission handed me a protest signed by a number of Republicans in my own ward saying "Take this and have some fun out of it. I would have reappointed you if every Republican in the county had signed it." I did have some fun out of it.

One Republican, Governor Bliss did ask me if I voted for Bryan or McKinley. I told him that I had voted for McKinley. He took up his pen to sign my commission but I held his hand and said "Governor there is one more question you should ask me. Did I vote for you?" I did not." Laughingly he said "I did not ask you that question," and signed the paper. Repeatedly I went before the legislature asking appropriations for the State Board of Health and the university and I never felt the least embarrassment on account of the political party to which I was accredited. Michigan Republicans at least those with whom I had to deal regarded a man's political affiliation as a personal right. Possibly then magnanimity might be accounted for by the fact that during most of this time Democrats in Michigan have been so few that they have been negligible.

The Michigan State Board of Health was fortunate in having for its secretary and executive officer for so many years Dr Henry B Baker. I have never known a man more thoroughly devoted to his work than he. To him the health of the people of the state was meat, drink and raiment. In it he forgot self and every other interest. Moreover, his devotion was not blind, nor actuated by sentiment but intelligently directed. Dr Baker deserves high rank among that small group of American pioneers in preventive medicine who set in motion the machinery by which human life has been greatly prolonged and human suffering greatly reduced. The present generation is enjoying the fruits of their labors and should not forget those to whom they owe these blessings.

When the Board was organized by act of the legislature of 1871, kerosene lamps were generally used in the illumination of houses, and frequent explosions destroyed in the twinkling of an eye, as it were, both property and lives. Doctors Baker and Kedzie went to work at this problem in a practical and scientific way. They discovered methods of determining the burning and flash points of kerosene and thus enabled the state to exclude from the market dangerous illuminating oils. Then they took up the matter of the resuscitation of the drowned and devised a method which is still superior to all others. For many years this was known as the "Michigan method." Recently it has been rediscovered by Professor Schafer of Edinburgh and is known under his name. The two methods are practically identical, even in detail.

Dr Baker made an early contribution to the world-old problem of the effect of weather upon disease. He showed that in Michigan at least the

death rate from pneumonia goes up as the mercury in the thermometer goes down. This does not mean that this rate is invariably higher in cold than in warm regions, but it does mean that in a given place the pneumonia death rate increases as the temperature falls. Of course temperature is only one of the factors in the cause and spread of pneumonia. In the early '80's some one suggested that salted food might be a factor in the causation of pneumonia. Dr. Baker decided to put this theory to a test and asked my assistance. Inasmuch as these experiments have never been published I will give a brief summary. We secured six fine monkeys. These were kept for some weeks in an air tight compartment, all the air entering being passed through sterilized cotton. The temperature of the air in the cage was kept constantly at about 80° F. The food consisted solely of milk and fruit without addition of salt. Suddenly the animals were transferred to a similar box in which the temperature stood below 10° F., kept there for from two to six hours and returned to the warm box. Not one showed any ill effects. Then they were kept for three weeks in the warm compartment and fed upon milk and fruit heavily salted. Exposure in the cold air was repeated and again without any evidence of pneumonia. Apparently, increase in the salt in the food did not predispose to pneumonia. I should add that these animals did slowly develop arthritis, but the salt in the food probably played no part in this.

The Michigan State Board of Health has been from the first an advisory and not a legislative or mandatory organization. It has sought to educate the people in sanitary matters and not to enforce its teachings by legal enactments leaving the latter to the initiative of the people as they advanced in knowledge. The value of isolation and disinfection in the infectious diseases was shown by comparative statistics in communities where these measures were and were not practiced.

Early in its existence the Board asked the legislature for an annual appropriation for the holding of sanitary conventions in different parts of the state. These were held for many years and did much to enlighten the people. The intelligent members of each community in which these conventions were held attended. The audiences were generally small but they made up in quality for what they lacked in numbers. The doctor who did not attend was likely to be faced by embarrassing questions from his patients who did. Lawyers and preachers took an interest in the matter spread the gospel and prodded their village and city authorities into action. This was many years before the university provided extension lectures. The time was much more propitious for this form of instruction than the present. The automobile, the graphophone, the movie and the radio were still unknown and even so serious a matter as a talk on public health was a welcome diversion. Besides, now, even public health talks are being overdone and every crank is airing his views and advertising his wares under this title. In my opinion, the Federal Government, the states, universities and certain benefactions are squandering thousands of dollars annually in so called public health courses, child welfare and health demonstrations. When the Michigan legislature of 1915 appropriated \$100,000 for the State Board of Health to use as it saw fit in the combat against tuberculosis wise men from the east hurried to the

west to tell us how to spend this money They were ready to supply us with so many speakers at so much a day to tell the people of Michigan that it had been demonstrated that tuberculosis is a contagious and therefore a preventable disease, that Koch had isolated the causative agent, and that disinfection of the sputum of tuberculous patients should be practiced The Board thanked these wise men for their altruistic interest in the matter, but informed them that every intelligent man, woman and child in Michigan was already aware of these facts and that the Board would start a traveling clinic through the state to find out about the prevalence of the disease and help the physicians in its recognition and prevention Because a procedure was wise and beneficial in the '80's is no proof that it is suitable at the present time

As I have indicated the Michigan Board, founding its teachings on the unimpeachable work of Villemin in the sixties, began preaching the contagiousness of tuberculosis years before Koch discovered the bacillus In doing this, quite naturally came an enumeration of the measures necessary for the restriction of the disease This accounts for the marked fall in the death rate from this disease in the state in the past forty years, so marked has this been that one enthusiastic statistician has been led to predict that the region of the Great Lakes may be the first part of the nation from which tuberculosis will disappear What we now need above all things for the protection and betterment of the health of the people is the establishment of a health center in every county, consisting of a hospital with a diagnostic laboratory, and the whole under the management of a staff of experts skilled both in preventive and curative medicine Such a health center should provide an ample library with standard books of reference and current scientific journals Every progressive city already has such facilities more or less developed but all in progress of growth When similar conditions are provided for rural communities there will be less need of the cry "Back to the farm" The lure of country life will call to many and not in vain The ideal land is that in which each citizen owns his own home and dominates his own affairs, so far as the rights of others are not abridged

In his desire to educate the people in health matters, Dr Baker (1895) framed a bill providing for instruction in all public schools in the nature of each infectious disease and the avenues through which it may be transmitted The publications in which this instruction is provided are supplied to the teachers by the Board Of course, generations must pass before the full effects of this provision can be reached, but it means progress However, all progress is liable to many jolts and not infrequently there is retrogression I do not dream that the people of Michigan or any other state are nearing the sanitary millennium, and I am fully aware of the fact that there is a limit below which the death rate from disease is not likely to fall Then, there will always be new methods and devices for killing off the population The automobile is now more deadly than smallpox and in the number of murders we are equalled only by our sister republics of the south with a fair chance of our winning the cup

In the '80's cases of poisoning from cheese, ice cream and other milk products became so numerous not only in Michigan but in adjacent states

that a search for the cause was amply justified. The Board instituted this investigation and the bulk of the work fell upon me and my laboratory helpers. In its prosecution I had the aid of the cheese makers especially that of Mr. Horton of Lenawee County, who put his factory at my disposal. An inspection of the dairies supplying this factory with milk furnished the clue. In many of these the cows were plastered with dung and other forms of filth. The animals were not submitted to even a pretense of cleaning. From unclean udders with filthy fingers the milk was drawn into unclean receptacles. There was no thought of keeping the milk cool. It stood for hours in the barn and the cans were carried often in the hottest season and during the hottest hours of the day to the factory. Here these cultures, containing the bacterial flora of the neighborhood were poured into a common vat in which bacterial growth continued under optimum conditions. Small wonder that cheese and ice cream made from these cultures should prove poisonous. Rules were drawn up for dairy inspection for cleaning the animals, for attention to the hands of the milkers, for sterile receptacles, for cooling the milk before and during transportation etc. Poisoning from cheese ice cream and other milk products became rare and soon ceased so completely that some say the whole thing is a fairy story and never occurred.

The Board backed by the Michigan Business Men's Association memorialized the Board of Regents of the University to ask the legislature of 1887 to make an appropriation of \$40,000 for the building and equipment of a hygienic laboratory at the university. The purposes for this request were stated in the following order: (1) research into the causation of disease, (2) the examination of suspected waters and foods on the request of health officials, (3) instruction of students in bacteriology. The Regents of the university reluctantly complied with this request from the Board of Health and put this item in their request for appropriations. The bill passed the legislature, was vetoed by the Governor and then passed over the veto. Thus was established the first hygienic laboratory in this country and the second in the world—the one at Munich, under the direction of Professor Pettenkoffer being the first. I wish to make the plain statement that this laboratory owed its existence to the State Board of Health and not to the Regents of the University. This appropriation was expended in the erection and equipment of a building in the rear of the library the basement and first floor were devoted to physics while the second floor and the attic were allowed for the purpose for which the appropriation was designed by the legislature. This building was known to collegiate students as the physical laboratory and to the medical students as the hygienic laboratory. When the new medical building was occupied in 1903 the hygienic laboratory and its work were transferred to it. I was made director of the hygienic laboratory and began to carry out the purposes as mentioned in the memorial of the Board of Health. In this work I had the most valuable assistance of Dr. Novak who has since become director of the laboratory. Samples of suspected water and food came in great numbers. During the following ten years reports including descriptions of the bacteria both harmless and harmful, found in 700 suspected waters, were made. It was in one of these reports that I coined the

imported from Europe, and were giving to the medical students the first comprehensive course in bacteriology offered in the United States, our laboratory became the *fons et origo* of all kinds of spooky stories, some quite surpassing in their hair-raising potency those told me of "raw-head and bloody bones" by black mammy in the old Missouri home. Even the dissecting room lost its pre-eminence in this particular. Mothers frightened their rebellious offspring into seemingly good behavior by threats of exposure to the hungry germs in the laboratory. Older children in crossing the campus gave our building a wide berth. Students in other departments shunned us. Learned professors looked at us with suspicion. The professor of physics whose laboratory was beneath us complained of the handicap under which he labored. One day an expressman in bringing a large carboy of suspected drinking water up to us, stumbled on the first floor, and delivered his burden short of its proper destination. Shrieks of terror from below brought me to the stairway where I saw the heels of fleeing professors, assistants and students. To add to the joy I shouted "Every drop contains a million typhoid bacilli." There was no return until my janitor had mopped up the water and scrubbed the floor with bichlorid. Some days later I was summoned to appear before the august Board of Regents. I faced nine grave-looking men and was solemnly asked if I was not endangering the lives of collegiate students working in the physical laboratory beneath mine. In my reply I tried to be humorous—a fault I seldom display. I said "Your question implies a great compliment. Dr. Novy and I and our students work in the midst of bacteria and you express no solicitude for our health and lives. There must be a divinity that throws a protective mantle about the person of a young man when he graduates in the college and enters the medical school."

So far as I know there were only two cases of accidental infection in the laboratory. I was inoculating guinea pigs with a typhoid culture while my assistant, Dr. Wheeler, held the animals. A drop fell from the syringe on her hand. Both of us saw it and she promised to sterilize her hands when we were through. We proceeded with our task and both forgot the drop until ten days later when she developed a mild attack of the disease. Of course the bacilli did not penetrate the skin but found their way into her mouth. There have been many accidental infections with this bacillus in other laboratories and none, so far as I know, have proved fatal.

The other instance was more serious. A young doctor, not a student, and supposedly already well grounded in laboratory technique, wanted to work with the plague bacillus. Our culture of this organism was old, attenuated and quite nonvirulent but would serve his purpose, so it was given him and he was assigned to a room in which he could prosecute his investigations which were to be continued indefinitely. During this time the Surgeon-General of the United States Public Health Service sent Doctors Novy, Flexner and Barker to San Francisco to study the plague. On his return Dr. Novy brought fresh virulent cultures of this bacillus. The young doctor purloined one of these and through faulty technique became infected. Doctors Novy, Dock and I spent some anxious days in caring for this patient who

During the eighties and early nineties a bacteriologic laboratory was regarded by the uninitiated with more respect and fear than a menagerie of wild beasts. At Hagenbeck's show one is protected from the lions and tigers by their cages and the iron railings but what protection can there be from invisible germs that are supposed to be floating through the air, seeking entrance to one's body through the mouth, nose, eyes, ears and even through the unbroken skin and ready to feed upon one's tissues bringing disease and death? I had many annoyances and occasional fun out of this phobia.

In 1888 I crossed the ocean from Bremen to New York on the *Lahn* with a wire basket full of a choice collection of pathogenic bacteria. Indeed, I had everything complete for the opening of the new laboratory in the fall. Koch's laboratory and the Pasteur Institute had denied Dr. Novy and me nothing. My stateroom chum was a university colleague Professor Denison of the engineering department. He had examined my sealed tubes and had convinced himself that no harm could come unless the tubes should be broken. He occupied the lower and I the upper berth. We anchored the basket to the sofa and deemed ourselves quite secure. One night in a storm he called to me that the twine holding the basket had broken, that the tubes were being tossed from one side of the room to the other and that we would be infected with Asiatic cholera before morning. I was seasick, he was not. I replied that Asiatic cholera would be a relief to me and that I would not crawl down to save myself from any or all infections. He, brave fellow that he was, secured the basket with strips taken from his valise and I was saved not only my prize collection but from paying useless tribute to Neptune.

In 1891 Mrs. Vaughan and I landed at Liverpool with the same basket. The inspector of customs paid but little attention to our trunks and valises and stamped his approval, but the basket was too much for him. The more I labored to explain the more suspicious did he become. He hinted rather plainly that we looked like Fenians—whatever these beasts might be—on our way to London with the intention of dynamiting Westminster and the houses of Parliament or horrors our destination might be Windsor and our intention to expedite the translation to Elvsum of that good old German woman who then ruled the empire. The inspector sought the counsel of his superior and he, in turn, until we had the whole customs force about us while we saw ourselves interned in the Tower of London and finally expiating our guilt after the manner employed by Henry VIII in getting rid of his numerous wives. Finally, one man more widely versed in current literature than the others solved the riddle by saying, 'Oh, I know, them are them Koch things.' The basket was stamped and we were soon complacently dining in a good English hotel.

Much of the reluctance exhibited by the authorities in accepting the appropriation for the laboratory was due to their fear of the germs. Of course, their solicitude was not for themselves but for the students under their guardianship. This was openly expressed a few years later when I proposed that an effort be made to secure the location at Ann Arbor of the State Sanatorium for Tuberculosis provided for by the legislature. When in the fall of 1888 Dr. Novy and I had stored away our dangerous germs

imported from Europe, and were giving to the medical students the first comprehensive course in bacteriology offered in the United States, our laboratory became the *fons et origo* of all kinds of spooky stories, some quite surpassing in their hair-raising potency those told me of "raw-head and bloody bones" by black mammy in the old Missouri home. Even the dissecting room lost its pre-eminence in this particular. Mothers frightened their rebellious offspring into seemingly good behavior by threats of exposure to the hungry germs in the laboratory. Older children in crossing the campus gave our building a wide berth. Students in other departments shunned us. Learned professors looked at us with suspicion. The professor of physics whose laboratory was beneath us complained of the handicap under which he labored. One day an expressman in bringing a large carboy of suspected drinking water up to us, stumbled on the first floor, and delivered his burden short of its proper destination. Shrieks of terror from below brought me to the stairway where I saw the heels of fleeing professors, assistants and students. To add to the joy I shouted "Every drop contains a million typhoid bacilli." There was no return until my janitor had mopped up the water and scrubbed the floor with bichlorid. Some days later I was summoned to appear before the august Board of Regents. I faced nine grave-looking men and was solemnly asked if I was not endangering the lives of collegiate students working in the physical laboratory beneath mine. In my reply I tried to be humorous—a fault I seldom display. I said "Your question implies a great compliment. Dr. Novy and I and our students work in the midst of bacteria and you express no solicitude for our health and lives. There must be a divinity that throws a protective mantle about the person of a young man when he graduates in the college and enters the medical school."

So far as I know there were only two cases of accidental infection in the laboratory. I was inoculating guinea pigs with a typhoid culture while my assistant, Dr. Wheeler, held the animals. A drop fell from the syringe on her hand. Both of us saw it and she promised to sterilize her hands when we were through. We proceeded with our task and both forgot the drop until ten days later when she developed a mild attack of the disease. Of course the bacilli did not penetrate the skin but found their way into her mouth. There have been many accidental infections with this bacillus in other laboratories and none, so far as I know, have proved fatal.

The other instance was more serious. A young doctor, not a student, and supposedly already well grounded in laboratory technique, wanted to work with the plague bacillus. Our culture of this organism was old, attenuated and quite nonvirulent but would serve his purpose, so it was given him and he was assigned to a room in which he could prosecute his investigations which were to be continued indefinitely. During this time the Surgeon-General of the United States Public Health Service sent Doctors Novy, Flexner and Barker to San Francisco to study the plague. On his return Dr. Novy brought fresh virulent cultures of this bacillus. The young doctor purloined one of these and through faulty technique became infected. Doctors Novy, Dock and I spent some anxious days in caring for this patient who

fortunately recovered. This, so far as I know, is the only case of laboratory infection with this bacillus which has ended in recovery. The details of this case have been published.

The relation between the State Board of Health and the Hygienic Laboratory of the University of Michigan was abruptly broken in the first decade of the present century. It happened in this wise. Health officers and others who sent samples of suspected water and food were charged \$10 for each examination—to cover expenses. Some failed to remit promptly. It was my custom to report by telegram when a bad water was found. About the time mentioned, the secretary of the university, a village tradesman, selected by the Regents to manage the finances of the university and incidentally to dictate to professors, instructed me not to report on my findings until the fee had been paid. Thus, if typhoid was epidemic in a city and the health officer sent me samples from several sources and I found one or more infected I could not report until the said health officer had remitted to the said secretary. Naturally the disease did not wait while these transactions were in progress. I was shocked by the instructions received from the secretary and immediately conspired with my fellow members on the Board of Health in securing an appropriation for a laboratory at Lansing, where the analyses are now made and reports are sent without awaiting remittances. In fact the Board of Health now has a branch laboratory in the Northern Peninsula. I would like to say more about this secretary but I am restrained by the old Latin proverb which may be translated thus: "Say nothing about the dead unless it be good."

The hygienic laboratory of the University of Michigan continues its work in studying the causation of disease and in giving instruction to students under the wise directorship of Dr. Novy, whose valuable contributions are well known to the scientific world. The examination of samples of water, food and other infected material on the request of health officers has been transferred to laboratories conducted by the State Board of Health. The efficient state health commissioner, Dr. Ohn, doing his work most creditably. Possibly my instructions from the secretary of the university, so odious to me at the time, have resulted not so badly after all, since the laboratory at Ann Arbor has been relieved of routine work and given more time for research.

RESOLUTIONS

THE FACULTY OF THE MEDICAL SCHOOL OF THE UNIVERSITY OF MICHIGAN

WHEREAS, after the lapse of nearly a decade death has again claimed an emeritus member of this Faculty, Victor Clarence Vaughan, who for forty-six years was an active teacher in this school and who for thirty years was its Dean

Eminent as a teacher, investigator, and administrator, he rendered most valuable service as a pioneer in the cause and progress of medical education and preventive medicine. He made many notable contributions to science. As an administrator he was conspicuous for his broadmindedness and justness. While insistent upon original productivity, he always accorded academic freedom to his colleagues.

Resolved, That the Faculty of the School of Medicine of the University of Michigan hereby express their realization of the great loss sustained in the passing of Victor Clarence Vaughan and their deepest appreciation of the services he rendered to the Medical School and to the University.

Resolved, further, That a copy of these resolutions be spread upon the minutes of the Faculty and that an engrossed copy be sent to the family

*F G Novy,
G Carl Huber,
A S Warthin*

January 6, 1930

THE RESEARCH CLUB

ANN ARBOR

WHEREAS, the Research Club of the University of Michigan has learned with profound sorrow of the death of one of its founder members, Professor Victor C. Vaughan, who was, during the first four years of the Club's history, its honored president, and whereas he maintained for the Club throughout the remainder of his lifetime an undiminished interest in its welfare

Therefore be it resolved that the Club herewith expresses its deep sense of the loss which it has sustained through his death, and furthermore, that this expression of his fellow members be entered upon the minutes, and that a copy of this resolution be transmitted to his family

*Preston E. James,
Secretary*

Ann Arbor, Michigan,
December 3, 1929

THE SCIENTIFIC CLUB

ANN ARBOR

MEETING year after year, informally, as we do, we are like a large family, and it is a grief to have one of our members taken from us. Doctor Vaughan was one of the founders of the Club, and to him it owes much of its unique character. Throughout his residence in Ann Arbor he was a faithful attendant at the meetings, and the papers which he read were not only instructive but suggestive and stimulating. In the discussions he revealed breadth of knowledge and interest in subjects far removed from his chosen field of work. But it was in the 'Angang' that his geniality, his keen sense of humor, his idealism, his strength of character showed themselves most clearly. It was a delight to listen to him. Every member of the Club felt that Vaughan was his friend, one to whom he could go for sound advice and for sympathy in time of trouble. He was so human, so simple of bearing, that it was hard for us to think of him as a great man, and yet we knew that his students, colleagues, his many friends, the University, the City, the State, the Country, had all been helped and strengthened by his devoted service. Much that he accomplished was made possible by the tender care of an ideal helpmate for whom all of us who know her have an affectionate regard.

To her and to the family, we of the Scientific Club extend our sincere sympathy.

THE NATIONAL TUBERCULOSIS ASSOCIATION

RESOLVED, That the Board of Directors of the National Tuberculosis Association hereby records its high esteem of the many contributions of Dr. Victor C. Vaughan deceased to the campaign against tuberculosis in the United States and more particularly to the National Tuberculosis Association. As a pioneer in the field of public health, as a teacher and administrator and as a student particularly in bacteriology, chemistry and related subjects, Dr. Vaughan was of great service to the tuberculosis movement. His assistance during the World War in developing cordial relations between the National Tuberculosis Association and other agencies was of great value.

As President of the National Tuberculosis Association in 1919, he rendered signal service. His work in the development of the Michigan Tuberculosis Association and in the furtherance of that organization's work in the early days went far toward making the tuberculosis program in that State a success, be it further.

Resolved, That a copy of this resolution be sent to the family of Dr. Vaughan and others who might be interested in receiving it.

January 25, 1930

DR VICTOR C VAUGHAN, an Honorary Fellow of the New York Academy of Medicine, died on November 21, 1929 Dr Vaughan was widely known in the United States as a chemist, pathologist, epidemiologist and hygienist He was for over forty-five years connected with the Medical School of the University of Michigan, having worked in the Departments of Chemistry, Physiology, Therapeutics and Hygiene

An indefatigable worker in the laboratory, he published in the earlier days of chemistry important textbooks on physiologic chemistry and proteins

In the field of epidemiology, Dr Vaughan's study of typhoid fever in the American Army camps during the Spanish American War was a remarkable example of painstaking inquiry, which exposed the woeful lack of hygiene then existing in the American Army This remarkable investigation, made in association with Reed and Shakespeare, paved the way toward the important reorganization of the methods employed by the Medical Corps of the Army, which produced such remarkable results during the Great War

During a period of fifty years of active life, Dr Vaughan was chemist, physiologist, pathologist, epidemiologist, teacher, soldier, scholar and scientist He was a constant advisor in the fields of medical education, public health and preventive medicine, much beloved by his students and associates, and recognized throughout the United States and abroad as well, as one of the most prominent scientists in the medical profession Be it therefore,

Resolved, That the New York Academy of Medicine hereby records its deep sense of loss in the death of Dr Victor C Vaughan, an Honorary Fellow of this Institution, whose services to humanity have been of enormous benefit and whose example will always be inspiring to those engaged in the relief of suffering and the prevention of disease, and be it further,

Resolved, That a copy of this Minute and Resolution be published in the Bulletin of the Academy and sent to the members of his family

December 5, 1929

DR VICTOR CLARENCE VAUGHAN
1851 1929

Presented to the Academy of Medicine
Richmond, Virginia, May 13, 1930

TO HIS ancestry and to his earliest environment Doctor Victor Clarence Vaughan attributed those qualities in his personality and in his character which fitted him for his life work. The blood of pioneers was in him. His forbears had lived in Virginia and in North Carolina in the early days. They were plain, honest, industrious, courageous folk, unafraid of the rough ways of frontier life. Of such ancestry he was born on the twenty seventh day of October, 1851 in the State of Missouri. That state was then peopled by frontiersmen and its civilization at that time must have been that of pioneer life.

While still a child he became acquainted with the horrors of warfare as the contending armies of the Civil War rolled back and forth across that border settlement. The poverty of the reconstruction period made exceedingly difficult the acquisition of an education. But in spite of hard conditions he completed the academic course in a college in his native state. In this college he then taught Latin to others and at the same time chemistry to himself. He pursued postgraduate work in the University of Michigan which led to the degree of Doctor of Philosophy. While still a student in this University he became a member of its teaching staff and at the same time a matriculate in the department of medicine and in 1878 he was graduated as a Doctor of Medicine. From that date until his resignation in 1921 as dean of the department of medicine Doctor Vaughan was continuously a member of the faculty of the University of Michigan. Although he was actively engaged in teaching from the time he reached adulthood until he was more than seventy years of age he was essentially an investigator engaged in clearing away medical ignorance and mysticism and he was aggressive always in his efforts to enable the practitioner of medicine to be a scientist. He did not permit his interest in other activities to prevent him from engaging actively in the practice of medicine. In this respect he set a good example for all those interested in research work.

The medical philosophy of Doctor Vaughan was simple and direct. He believed that sickness is due largely to the introduction of poisonous substances into the body. If the introduction of such poisons could be prevented or their toxic effect upon the body neutralized the human race would be healthier and individual death would be deferred. In consequence of such an attitude Doctor Vaughan early in life became interested in the toxic effect of certain chemicals, and his subsequent medical philosophy was but a broadening of that simple conception. As a young physician and teacher he was not only enormously interested in the new germ theory of disease but he went

abroad for study, acquired all the knowledge that could be gotten in the laboratories of Europe about the living microscopical causes of disease, and he promptly equipped at the University of Michigan probably the first bacteriological laboratory in the United States. In that University he taught not only bacteriology, but in succession histology, materia medica and therapeutics, and public hygiene and sanitation. He gave to medicine modern toxicology and the basic factors in public health work. For about forty years Doctor Vaughan was president of the State Board of Health of Michigan. In that capacity he gave fully to the welfare of the people of the state the benefits of his great industry and learning. For thirty years he was dean of the Medical Department of the University of Michigan. There he not only taught scientific medicine, but he instantly assumed leadership in this field, and his zeal and his ability to sense developing genius in others enabled him to bring into that faculty the most alert minds in this country. Consequently the school of medicine of the University of Michigan became one of the leading medical schools in the world. Probably no other school in the United States has given inspiration and sound training to so many medical teachers.

Doctor Vaughan was born soon after the close of the Mexican War. He was an actual participant in the Civil War, the Spanish-American War, and the World War. In the border state of Missouri neighbors were divided against neighbors, and as a boy he experienced all the physical and spiritual suffering of border warfare. He served as a medical officer throughout the war with Spain. During that brief but tragic clash he ministered to the wounded and the dying under fire, and only a little later he all but succumbed to an attack of yellow fever. With Shakespeare and Walter Reed he served on a Commission to investigate the ravages of typhoid fever amongst the soldiers of the United States army. The practical disappearance of that disease is due largely to the knowledge acquired by that Commission. The deaths of Reed and Shakespeare placed upon Doctor Vaughan the labor and the responsibility of formulating that voluminous and historic work. Doctor Vaughan had prophetic knowledge of our eventual participation in the World War, and he gave himself freely as a commissioned medical officer in that great struggle. Although he had reached the age of almost seventy years it is to be doubted if any other physician rendered more effective service during that period. Yet he hated warfare, and military trappings and the pedestalization afforded by rank made no appeal to him. It is said that toward the end of the World War he declined to accept a proffered promotion to the rank of Brigadier-General.

Few medical men have made more helpful contributions to medical literature than Doctor Vaughan. He believed in giving the benefit of the newest medical knowledge to all the people through the ministrations of the family doctor and the activities of public health organizations. His volumes, half a score of them, occupy positions of helpfulness in every well-stocked medical library. And he must have contributed almost 200 theses and monographs to sound medical thought. He was the first editor of *Hygiene*. After his retirement from the deanship he published his autobiography—*A Doc-*

tor's Memories American literature does not afford a more appealing, delightful, and informative account of a brave useful and unselfish life than that of this energetic and versatile physician

He was interested in organized medical effort only as an agency in behalf of human welfare and he occupied high office in most of the national organizations but he thought in terms of disapproval of medical politics and he never sought position for himself

Doctor Vaughan was blessed in his home life In 1877 he was married in Missouri to Dora Catherine Taylor, whom he had known since their childhood Of the five sons four became physicians all enlisted for service in the World War and one of the sons gave his life to his country After his retirement from active work Doctor Vaughan came to Richmond to spend his last days near his son, Dr Warren T Vaughan, a member of this Academy We honored ourselves by inviting Doctor Vaughan into honorary membership in this body No more distinguished physician has ever lived in the Commonwealth of Virginia His sudden death on November 21, 1929 was preceded for some time by considerable impairment of health but his vigorous mind never became inactive and his splendid courage suffered no abatement

It is to be doubted if Doctor Vaughan would have laid claim to the possession of any spark of genius He was an advocate of simple living intellectual honesty unwearied industry and public service The poor obscure, industrious courageous Missouri country lad made of himself one of the great physicians of the world, and one of the most beneficent servants of mankind His career should be an incentive to all aspiring youth And to the ageing his latter days should serve as a good example The quality of greatness was in his attitudes and upon his achievements in the domain of medicine there is the impress of immortality But he looked upon himself only as an humble coworker with the Creator of the Universe

Respectfully submitted

Dean B Cole
C C Coleman
Stuart N Michaux
Jas H Smith
Jas K Hall

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CLINICAL AND EXPERIMENTAL

A STUDY OF THE BLOOD UREA CLEARANCES WITH RELATION TO DIURESIS IN NORMAL AND NEPHRITIC ANIMALS*

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THE purpose of this investigation is to show the close correlation which exists between the diuresis curve in the fasting mammal and the curve of blood urea clearance. Certain important papers are briefly reviewed below, these serve as a basis of comparison, as well as to explain the terminology used.

Investigations by Møller, McIntosh, and van Slyke¹ confirm those of Marshall and Davis, Pepper and Austin,² Addis and Watanabe,⁴ that when the volume of urine is fairly large the rate of urea excretion is directly proportional to the blood urea content. They call attention to the fact that Austin, Stillman, and van Slyke⁵ demonstrated in three normal subjects that the rate of excretion holds only when the urine volume is above a certain limit (about 2 c.c. per minute in adults) which they called the "augmentation limit." When the volume of urine became less than the so-called augmentation limits of the subjects studied, the urea output decreased in proportion to the square root of the volume. This conception is confirmed by Møller, McIntosh, and van Slyke on seven other human subjects. Austin, Stillman, and van Slyke⁵ considered that a further increase in the volume of urine would not result in a further increase in the hourly output of urea.

Møller, McIntosh, and van Slyke¹ stated, furthermore, that in adults when the excretion of urine is proceeding at the rate of 2 c.c. or more per minute, a certain volume of blood will be freed of urea each minute. They found that in men with normal kidneys this volume of blood was approxi-

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mately 75 cc per minute, and called this figure the maximum blood-urea clearance, designated C_m . They found also that in men this maximum clearance is practically a constant figure for all urine volumes equal to or above the so-called augmentation limit (of about 2 cc per minute). Under conditions of diuresis, however, in which the urine excretion fell appreciably below 2 cc per minute, the amount of blood which was cleared of urea diminished, and another basis of calculation was necessary for such instances. It was found that as the volume of urine diminished more and more, the diminution of the number of cubic centimeters of blood cleared of urea per minute was not exactly proportional to the volume of urine, but was more nearly proportional to the square root of the volume when this volume was below the augmentation limit of 2 cc per minute. For such conditions, the authors coined the term "standard clearance," which they defined as *the efficiency with which the kidneys excrete urea when the volume of urine is at the average normal level of 1 cc per minute (or 1,440 cc per twenty-four hours)*, designated as C_s . The formula for standard clearance, as derived by these authors is

$$C_s = U/B \sqrt{V} \quad (1)$$

For maximum clearance, the formula is

$$C_m = UV/B \quad (2)$$

U designates the milligrams of urine urea per 100 cc, B the milligrams of blood urea per 100 cc, and V the volume of urine per minute.

As already stated, the second of these formulae was used for adults only when the urine volume was 2 cc or more per minute. For adaptation of the formulae to extremely heavy or extremely thin adults, or to children, the authors recommended that the value for V (urine volume) be multiplied by the formula introduced by Addison

$$1.73/\text{Sq Meters Surf Area} \quad (3)$$

A complete discussion of the use of this formula is given by McIntosh, Moller, and van Slyke.⁵ These authors are in accord with MacKay and MacKay⁷ that loss of renal function may exceed 60 per cent before the blood-urea content rises above the highest level observed in normal subjects, and they state that unless the excretion rate is also considered, the blood urea may fail to reveal diminishing renal ability until the latter has become advanced.

Eaton M. MacKay⁶ observed that urine volume was not the only important factor affecting urea excretion. He investigated the diurnal variation of the standard blood urea clearance and found this variation to be considerable in each individual under normal conditions, and that a further variation is brought about by exercise. His experiments indicate that there is less variability in a series of observations made on an individual at the same hour on different days than in a series of observations made on the same day.

EXPERIMENTAL DATA

Before undertaking this study on dogs, we conducted 28 experiments in diuresis on a fasting subject with normal renal function under varying conditions of activity. Some of these experiments were done with the subject

in bed, others while doing sedentary work, and still others during the performance of mild exercise. Similar diuresis tests were conducted on four other healthy subjects and four with nephritis determined by at least two criteria in each case. The experiments done on fasting human subjects differed from those done on dogs in that the human beings drank the two 500 cc portions of water, and urine specimens were passed voluntarily, a procedure which is satisfactory when there is no pathologic condition causing urinary retention. In all of the 32 tests on nonnephritic human subjects the diuresis curves were typical of those obtained in normal dogs, there being a high peak of excretion within two hours, with a subsequent abrupt decrease of the excretion rate to a point near the fasting level within five hours. The curves in nephritic human subjects, however, lacked the normal high peak

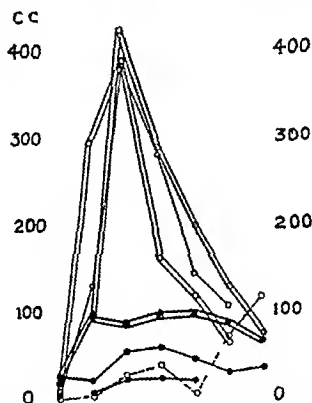


Fig 1—Curves of water diuresis hourly specimens in normal and nephritic animals. The higher, open circle curves are of nonnephritic animals. The lower black dot circles are of nephritic animals. The double line curve in heated results following administration of 1000 cc of 5 per cent urea, the single line curve following 1000 cc of water and the dotted line curve following 1000 cc of 5 per cent urea and 1 cc of surgical pituitrin.

within the first two hours and failed to reach an equilibrium within five hours. Such tests are distinctly helpful, but are not intended to replace studies of the blood chemistry.

Our observations of standard and maximum blood urea clearances in dogs were made both before and after the induction of acute nephritis by means of uranium acetate, 25 mg per kilogram subcutaneously. Fig 1 shows the results in graphic form. Fig 2 shows graphically the urine volumes noted in the tables. The bases of computation of these blood urea clearances are given in Tables I to VII. (A rapid survey may be made of these tables by reading the extreme left and right hand columns together.)

It is noted that when large doses of urea are given to dogs with normal renal function the absolute amounts of urea excreted and the volume of urine are very greatly increased. This mechanism may serve as a rapid means of dehydration. The number of cubic centimeters of dog blood cleared

of urea per minute is definitely decreased in most instances, both in the normal and in the nephritic animal, but in our hands the decrease was negligible if pituitrin was given at the same time as the urea

The designation *maximum blood-urea clearance* which has been applied to man is a misnomer for the dog McIntosh, Moller, and van Slyke conclude that about 75 cc is the average quantity of blood which can be cleared of urea per minute in healthy men when the urinary output is 2 cc or more per minute, and it is inferred that this, generally speaking, is the maximum

TABLE I

HOUR OF EXPERIMENT	URINE VOLUME, CC PER HOUR	URINE VOLUME, CC PER MIN	SQ RT OF VOL PER MIN	URINE UREA, GM PER 100 CC	BLOOD UREA, GM PER 100 CC	URINE UREA, GM PER HOUR	BLOOD UREA CLEARANCE, CC PER MIN
control	65	0.10	0.33	4.77	0.024	0.310	65 (standard)
1	7.0	0.11	0.33	3.51	0.021	0.245	55 (standard)
2	36.0	0.60	0.78	2.58	0.027	0.928	74 (standard)
3	44.0	0.73	0.85	2.34	0.021	1.029	94 (standard)
4	30.0	0.50	0.71	2.28	0.024	0.684	67 (standard)
5	73.0	1.30	1.14	1.14	0.060(?)	0.889	—
6	122.0	2.03	1.42	0.60	0.033	0.732	26 (standard)

Dog No 3, normal, before induction of nephritis The table shows the basis of computing the hourly blood urea clearances following two doses of 500 cc each of water only, given by stomach tube, and 1 cc of surgical pituitrin given subcutaneously

TABLE II

HOUR OF EXPERIMENT	URINE VOLUME, CC PER HOUR	URINE VOLUME, CC PER MIN	URINE UREA, GM PER 100 CC	BLOOD UREA, GM PER 100 CC	URINE UREA, GM PER HOUR	BLOOD UREA CLEARANCE, CC PER MIN
control	33.5	0.56	3.84	0.027	1.286	106 (standard)
1	102.0	1.70	2.73	0.207	2.784	22.4 (maximum)
2	427.0	7.11	2.82	0.240	12.041	83.0 (maximum)
3	295.0	4.91	3.15	0.213	9.292	72.0 (maximum)
4	206.0	3.43	2.76	0.147	5.685	63.8 (maximum)
5	136.0	2.26	3.84	0.132	5.222	65.7 (maximum)
6	80.0	1.33	5.34	0.120	4.272	57.8 (maximum)

Dog No 3, normal, before induction of nephritis The table shows the basis of computing the hourly blood urea clearances following two doses of 500 cc each of 5 per cent urea solution given by stomach tube, and 1 cc of surgical pituitrin given subcutaneously

TABLE III

HOUR OF EXPERIMENT	URINE VOLUME, CC PER HOUR	URINE VOLUME, CC PER MIN	URINE UREA, GM PER 100 CC	BLOOD UREA, GM PER 100 CC	URINE UREA, GM PER HOUR	BLOOD UREA CLEARANCE, CC PER MIN
control	14	0.23	5.70	0.039	0.798	70.5 (standard)
1	302	5.03	1.80	0.330	5.436	28.8 (maximum)
2	395	6.58	2.07	0.249	8.176	55.0 (maximum)
3	172	2.86	3.30	0.231	5.211	40.0 (maximum)
4	123	2.05	4.59	0.204	5.545	45.0 (maximum)
5	74	1.23	5.40	not taken	3.996	—

Dog No 3, normal, before induction of nephritis The table shows the basis of computing the hourly blood urea clearances following two doses of 500 cc each of a 5 per cent solution of urea given by stomach tube It is to be noted that under stress of high blood urea, the kidney clears less blood per minute

amount of blood which can be cleared of urea during the most forced diuresis. Certainly this does not apply to dogs, and it is reasonable to expect a difference in view of the variation in surface area. This difference is further emphasized after the administration of large amounts of urea by mouth. Doubtless the results of diuresis tests are also affected by the plane of protein metabolism existing at the time of the test.

It seems very probable that the secretion of the pars intermedia (the active principle of pituitary extract) is an important factor in determining the output of urea, and should take its place as such along with other factors which influence the quantity of blood cleared of urea per minute, namely magnitude of urine volume, diurnal variation exercise and other conditions which alter the flow of blood through the kidneys. It is emphasized that

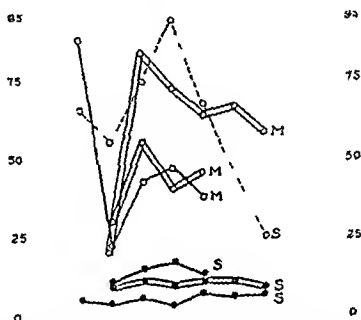


Fig. —Curves of blood urea clearances. Those marked *M* are based on the formula for maximum clearance; those marked *S* are based on the formula for standard clearance. The dosage corresponds to the line designations in Fig. 1. All charts are constructed from the tables.

while pituitary extract is known to alter the blood flow through the kidneys in a classical manner and at the same time is noted to diminish urine volume (Tables II and VI), the amount of blood cleared of urea per minute in these experiments does not share in this decrease but may actually be increased. Further work is needed in connection with these facts.

TABLE IV

HOUP OF EXPERI MENT	URINE VOLUME, CC PER HOUR	URINE VOLUME CC PER MIN	URINE UREA GM PER 100 CC	BLOOD UREA, GM PER 100 CC	URINE UREA GM PER HOUR	BLOOD-UREA CLEARANCE CC PER MIN
control	9	0.15	6.12	0.027	0.550	87.0 (standard)
1	137	2.28	1.71	0.192	2.330	20.4 (standard)
2	393	6.55	1.62	0.246	6.366	42.8 (standard)
3	287	4.78	2.31	0.234	6.629	47.0 (standard)
4	150	2.50	3.0	0.193	4.500	38.0 (standard)
5	115	1.91	3.48	not taken	4.000	—

Dog No 5, normal, prior to induction of nephritis. The animal was etherized, and one 500 cc dose of water was given immediately, a second 500 cc dose being given thirty minutes later. The table shows the basis of arriving at the standard urea clearances.

The curves of blood-urea clearance in the normal and nephritic animal and the type of curve of urine volume (with active diuresis), as given in Figs 1 and 2, should be compared. These curves begin with the fasting state, and are carried through the period of copious diuresis, hourly specimens being taken until equilibrium is practically restored.

TABLE V

HOUR OF EXPERIMENT	URINE VOLUME, CC PER HOUR	URINE VOLUME, CC PER MIN	SQ RT OF VOL PEP MIN	URINE UREA, GM PER 100 CC	BLOOD UREA, GM PER 100 CC	URINE UREA, GM PER HOUR	BLOOD UREA CLEARANCE, CC PER MIN
control	none obt	—	—	—	0.069	—	—
1	11.0	0.183	0.42	1.80	0.069	0.198	10.90 (standard)
2	28.0	0.466	0.68	1.35	0.060	0.378	15.07 (standard)
3	30.0	0.500	0.71	1.35	0.054	0.405	17.70 (standard)
8	24.5	0.408	0.64	1.11	0.051	0.271	13.80 (standard)

Dog No 5, nephritic, five days after the subcutaneous injection of uranium acetate, 25 mg per kilogram. The table shows the basis of computing the hourly standard urea clearances following two doses of 500 cc each of water given by stomach tube. The blood urea clearance has fallen from a pre-nephritic level of 47 (for the third hour) to 17.7 cc

TABLE VI

HOUR OF EXPERIMENT	URINE VOLUME, CC PER HOUR	URINE VOLUME, CC PER MIN	URINE UREA, GM PER 100 CC	BLOOD UREA, GM PER 100 CC	URINE UREA, GM PER HOUR	BLOOD-UREA CLEARANCE, CC PER MIN
control	25	0.41	1.35	0.108	0.337	—
1	100	1.66	2.04	0.330	2.040	9.9 (maximum)
2	95	1.58	2.43	0.321	2.308	11.9 (maximum)
3	105	1.75	1.86	0.303	1.953	10.7 (maximum)
4	105	1.75	1.86	0.282	1.953	11.1 (maximum)
5	95	1.58	1.86	0.273	1.767	10.7 (maximum)
6	72	1.20	2.19	0.264	1.576	9.95 (maximum)

Dog No 5, nephritic, seven days after the subcutaneous injection of uranium acetate, 25 mg per kilogram. The table shows the basis of computing the hourly (so called maximum) blood urea clearances following two doses of 500 cc each of a 5 per cent solution of urea given by stomach tube. Of the second dose, 440 cc was vomited, but an equal amount of water was promptly given by stomach tube.

TABLE VII

HOUR OF EXPERIMENT	URINE VOLUME, CC PER HOUR	URINE VOLUME, CC PER MIN	SQ RT OF VOL PEP MIN	URINE UREA, GM PER 100 CC	BLOOD UREA, GM PER 100 CC	URINE UREA, GM PER HOUR	BLOOD-UREA CLEARANCE, CC PER MIN
control	33.0	0.55	0.74	1.05	0.144	0.346	5.3 (standard)
1	26.5	0.44	0.66	0.93	0.126	0.247	4.8 (standard)
2	80.0	1.33	1.15	0.54	0.114	0.432	5.4 (standard)
3	65.5	1.09	1.05	0.48	0.108	0.314	4.6 (standard)
4	50.0	0.83	0.91	0.66	0.099	0.330	6.0 (standard)
5	38.0	0.63	0.79	0.87	0.126	0.330	5.4 (standard)
6	40.5	0.67	0.82	0.90	0.120	0.364	6.1 (standard)

Dog No 3, twelve days after the subcutaneous injection of uranium acetate, 25 mg per kilogram. The table shows the basis of computing the hourly standard urea clearances following two doses of 500 cc each of water given by stomach tube, one dose immediately after the control hour, the other dose thirty minutes later.

PRACTICAL APPLICATION OF THE TEST

In this connection one point in particular should be emphasized, namely that the majority of patients presenting themselves for diagnosis and treatment should have some evaluation of the renal status when it can be done so quickly and simply. The patient should be requested to come to the office after a twelve hour fast, bringing a specimen of the night urine and one taken an hour after the completion of the night specimen. On arriving, he can be seated to await his turn. When called he should be requested to empty the bladder, this specimen being discarded. He should then be given 500 cc of water to drink, and an additional 500 cc thirty minutes later. Beginning from the time of the first dose of water urine specimens should

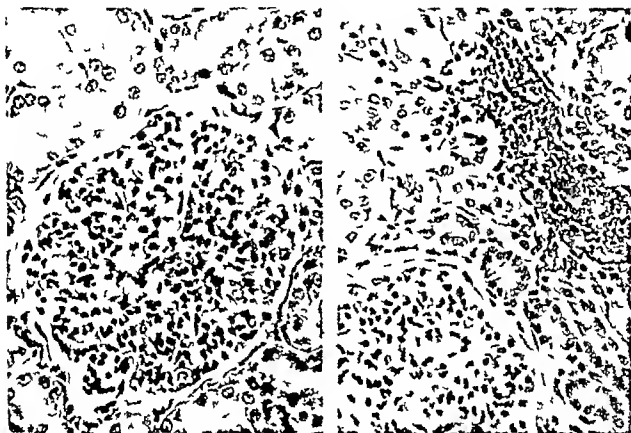


Fig 3—High power views of a kidney from dog No 3 nineteen days after the injection of uranyl acetate subcutaneously 2.5 mg per kg

be taken hourly for not more than six hours. The specific gravity and albumin content of the night specimen should be determined and the volume and specific gravity of the remaining specimens.

A very satisfactory normal type of curve is one which gives a high volume peak in the second hour and an abrupt falling off of volume in the third, fourth, and fifth hours. The specific gravity is very low in the second hour, but gradually rises and approaches that of the night specimen by the fifth or sixth hour. The difference between the specific gravity of the night specimen or the sixth hour specimen and that of the second hour specimen will give a good estimate of the ability of the kidneys to concentrate urea, and this finding will be borne out by a subsequent determination of urea concentration if it seems necessary. If there is no good reason to suspect kidney damage, further tests are not indicated.

If the curve obtained is within the average, the following conditions will also be found, with but exceedingly rare exceptions

- 1 The test of phenolsulphonephthalein excretion will be satisfactory
- 2 The output of urea will be normal
- 3 There will be no fixation of specific gravity
- 4 The blood-urea clearance will be normal
- 5 There will be no fixation of blood-urea nitrogen at a high level, and the blood-nonprotein nitrogen will be within normal limits
- 6 The functional capacity of the circulation at rest will be adequate for good renal function

If edema is present, a blood chloride determination is imperative. If this gives figures near normal, a sodium chloride tolerance test should be

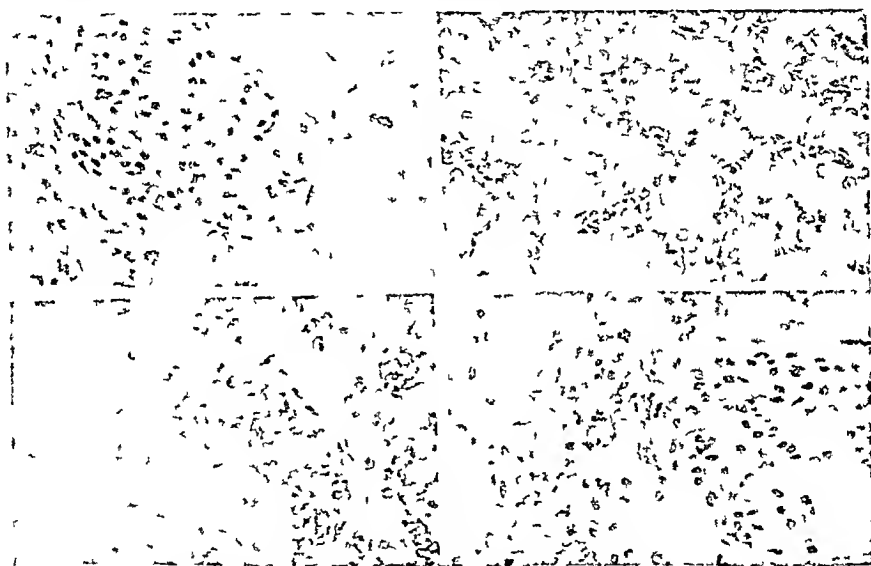


Fig. 4—High power views of a kidney from dog No. 5 seven days after the injection of uranium acetate subcutaneously 2.5 mg per kg

done, with parallel determinations of chloride on the blood and urine. If the chloride elimination is normal and chloride feeding does not cause untoward symptoms, the amount of this important element in the diet should not be limited or derangement of the acid-base equilibrium may result. If urea is tolerated well and eliminated normally, the diet should contain a fairly liberal amount of protein. In cases of nephritis with edema in which the patients tolerate urea well, Hugh McLean gives 15 gm of urea by mouth daily for several days, alternating with a similar period in which no urea is given. The dehydrating effect of the urea reduces the edema in some of these cases just as administration of urea produces dehydration in normal dogs.

If focal infection is known to have existed for a long time or if there is a high diastolic pressure, arteriosclerosis, headache, mental inertia, and spots

before the eyes—a syndrome suggesting renal injury—a further study of the renal efficiency is certainly indicated. This should include (1) some evaluation of the acid base balance such as determination at least of the carbon dioxide combining power of the plasma and the sodium chloride content of the plasma or of the whole blood, (2) an estimation of the fasting blood urea and (3) a study of the albumin and globulin fractions of the blood. The results of these tests added to those of the diuresis curves and phenolsulphonephthalein excretion, should either give an insight leading to a definite diagnosis or indicate the need for other more specialized renal tests. If the carbon dioxide combining power of the plasma is very low (35 or less) the patient should have glucose daily. When this is not tolerated by mouth (because of vomiting) it may be given intravenously with insulin if the sugar tolerance is impaired. As pointed out by Peters, Wakeman, Fisenman and Lee⁹ the glucose may save tissue protein from destruction and also diminish the ketosis.

Hospitals routinely force fluids on patients with nitrogen retention. A nephritic patient who receives three or more liters of fluid daily should have a determination of the corpuscle volume index to learn whether there is plethora or blood concentration, as an indication for decrease or increase of the fluid intake. Peters and his coworkers⁹ found that hyponatremia and a low serum base are usually attended by anhydremia and general dehydration, and pointed out that if the blood chlorides are low, the forced water diuresis (which is part of the therapy against toxemia and anhydremia) may further deplete the chlorides to a very harmful extent unless the patient is given sodium chloride in the diet to the extent of about seven to ten grams daily with a fluid intake of 2 000 to 3 000 c c.

These same authors⁹ report sixteen determinations of serum albumin and globulin in twelve healthy subjects which averaged 5.19 per cent for albumin and 2.1 per cent for globulin—7.29 per cent total protein. Rowe¹⁰ reports analyses in 22 cases with variations of serum albumin between 4.6 and 6.7 per cent, globulin between 1.2 and 2.3 per cent, total proteins between 6.5 and 8.2 per cent, with averages for serum albumin of 5.6 per cent, serum globulin 1.9 per cent, and total protein 7.5 per cent. This author used the method of Robertson.¹¹ In his review he reports definite increases in serum globulin in syphilis, and states that in pneumonia it is increased more in relation to the total protein than in syphilis, while the total protein is reduced. In many chronic septic conditions, he continues, in mild infections and in typhoid fever, the total protein is not decreased as it is in pneumonia while globulin seems definitely increased in all infections, except in acute tonsillitis, typhoid, and certain mild infections such as chronic bronchitis.

Howe¹² reviewed the physiology of the plasma proteins, and pointed out the conditions which may cause variation, such as species, age, water balance, activity, disease, etc., stating that under ordinary conditions the composition of the plasma of an individual is relatively constant. He considers that isolated determinations are of little value, and recommends a series

of such analyses following the course of the disease. With more general use of this test it may become increasingly helpful in the classification of nephritides.

SUMMARY

Data are presented correlating the water-diuresis curve with the ability of the kidneys to clear the blood of urea in normal and nephritic animals.

A simple diuresis test may be used in patients of limited means to indicate the results which may be expected from certain more extensive, expensive, and time consuming renal tests.

This research has been conducted under the James W. Pickard Research Endowment Fund. The blood chemical determinations were carried out by Miss Harriet Hippard, of the Department of Biochemistry, to whom thanks are extended. We desire also to express appreciation to Dr. John Tucker, of the Department of Medicine, for criticism of the manuscript.

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THE INCIDENCE OF LIPOIDS IN URINE*

BEING A REPORT OF THE MICROPOLARISCOPIC EXAMINATION OF 1470 SPECIMENS

By ARTHUR I. BRILL, JR., B. A., FLORENCE, S. C.

THE researches of von Noorden, Munk, Genck and Miloslavich¹ indicate the fact that in normal urine no anisotropic substances of lipoidal composition can be found. Miloslavich further emphasizes the experimental fact that during the course of an artificial hypercholesterolemia lipoids were found in the urine only after the kidney had been previously damaged with uranium nitrate. These facts completely differentiating the finding of lipoids in the urine from the normal cholesterol metabolism of the body is evidenced in the blood stream would seem to link them closely with the etiologic complex of the diseases in which they may be found.

Miloslavich has classified these diseases under the following heads:

- "1 Lipoid nephrosis of unknown origin (genuine lipoid nephrosis)
- "2 Lipoid nephrosis of syphilitic etiology (secondary stage of syphilis)
- "3 Subacute and chronic glomerulonephritis (combination forms of nephritis and nephrosis)
- "4 Amyloid nephrosis with edema
- "5 Grawitz tumor (so called hypernephroma) of the kidney
- "6 Lipoid carcinoma of the prostate"

He advises us that the micropolariscopic examination of a urinary sediment from these diseases may reveal the following morphologic elements:

"a A minute double refracting granule either isolated or found in groups (Spoken of in this paper as granule)"

"b A cast, consisting mainly of anisotropic material which is termed lipoid cast

"c Small epithelial cells, apparently desquamated cellular elements of the tubular apparatus of the kidney, which contain double refracting substances in their protoplasm (Spoken of in this paper as lipoid epithelium)

d Larger foamy cellular elements including anisotropic fat substances derived from blastomalous growths in the genitourinary tract (Spoken of in this paper as lipoid cysts)

In a private communication² Professor Miloslavich has further advised us that the exact chemical constitution of these elements is not well understood and that it is known to vary though the constant constituent is cholesterol.

Our investigations conducted over a period of about two years have placed us in a position to add but little to this classification. The typical

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large lipoid granule under the low (10×10) power of the microscope with crossed Nicols reveals a characteristic polarizing cross-figure, somewhat resembling a black Maltese cross. When small this cross-figure may be barely discernible under the high (10×40) power. We also believe that there occurs a tiny granule discernible under the high power, the morphology of which, described in two planes only, resembles four diamonds with acute angled points placed in close juxtaposition. We have definitely identified this morphology in two specimens. The lipoid granules are not soluble by boiling the specimen for several minutes, though this procedure seems to reduce their anisotropic property. They are not immediately soluble in equal parts of urine and any of the following reagents: (a) 3.5 per cent neoarsphenamine solution, (b) N/10 sodium hydroxide, (c) 5 per cent acetic acid, (d) 6 per cent sulphuric acid.

The lipoid epithelial cell is the most frequently found lipoidal element, having been observed in 41 per cent of our 152 positive specimens, or 5.5 per cent of our 1146 valid examinations. The anisotropic phenomenon exhibited in this element may at times be but very faintly pronounced, its degree depending undoubtedly upon the concentration of lipoid substances in the protoplasm.

At this point it might be well to state that for the purposes of our investigation one of the laboratory microscopes was equipped with Zeiss polarizing attachments, and the principles of procedure as laid down in Simon H. Gage's textbook were followed throughout. The selenite test plate was not used. Its use is to be recommended, however, in any further investigation or routine work, as it will render more positive the identification of but faintly anisotropic substances.

The lipoid cast can be differentiated from the common granular cast only on the basis of the anisotropic phenomenon by the use of polariscope attachments. The doubly refracting substance in general seems to be evenly diffused through the body of the cast, as it is in the lipoid epithelial cell, though we have observed a specimen in which the coarsely granular casts seemed to consist largely of small lipoid granules, the characteristic Maltese cross figure being distinctly visible under crossed Nicols.

The foamy elements, which we have designated as lipoid crystals, are quite characteristic in appearance. Under crossed Nicols they seem to contain spots of a much more highly refractive nature than the body of the element, the whole being of a somewhat cellular type of structure considerably larger than an epithelial cell.

We have also identified in a very small number of specimens, 3 per cent of the positives, less than 1 per cent of the total, an anisotropic pus cell. The greatest numbers of these were observed in two different cases of diabetes complicated by pyelitis, which suggests the possibility of the existence of a complex of this type.

In general our findings published in Tables I to IV, prove the truth of Mikolovich's assertions that doubly refracting lipoidal substances may be found in other conditions but are particularly characteristic of the degenerative diseases of the kidney. It has not been our opportunity to examine speci-

TABLE I
860 SURGICAL SPECIMENS

	NEGA TIVE	PER CENT	INVA SAD	PER CENT	GRAN ULES	PER CENT	CRYS TALS	PER CENT	CASTS	PER CENT	EPITHE LIUM	PER CENT	PUS	PER CENT	TOTAL POSITIVE	PER CENT
Appendectomy	262	85	67	18	12	4	9	3	2	7	22	—	—	—	45	15
Salpingectomy	33	70	7	22	2	4	—	—	1	10	8	2	1	—	12	24
Miscellaneous laparotomy	58	82	14	16	0	8	1	—	1	7	5	—	—	—	13	18
Tramatic cases	30	100	21	23	—	—	—	—	—	—	—	—	—	—	0	0
Infections	23	96	5	17	—	—	1	4	—	—	—	—	—	—	1	4
Kidney	12	100	—	—	—	—	—	—	—	—	—	—	—	—	0	0
Gall bladder	19	73	6	19	5	19	1	4	1	—	—	—	—	—	0	0
Thyroid	11	85	3	19	—	—	—	—	—	—	2	—	—	—	7	27
Tumors	23	82	3	10	1	4	—	—	—	—	4	—	—	—	2	15
Eye, ear nose and throat	38	97	0	19	1	3	—	—	—	—	—	—	—	—	5	18
Gynecologic	70	96	0	8	—	—	—	—	—	—	3	—	—	—	1	3
Gonourinary	12	92	4	24	—	—	—	—	—	—	1	—	—	—	3	8
Plastic (herniotomy)	12	90	4	21	3	20	—	—	—	—	—	—	—	—	1	20
Minor	15	83	5	22	1	6	—	—	—	—	2	—	—	—	3	17
Totals	629	86.8	144	17	31	4	12	2	5	6	47	—	1	—	96	13.2

TABLE II
501 MEDICAL SPECIMENS

	NOV TITR	LEU CENT	INVA TITR	LEU CENT	GRAN ULFS	LEU CENT	CRYS TALS	PER CENT	CASTS	LEU CENT	PLATEL TUM	LEU CENT	PUS	LEU CENT	TOTAL TITR	PER CENT
Acute infections	94	87	24	18	1	4	—	—	8	7	1	1	1	1	11	11
<i>Dysenteriae</i>																
Kidney	15	71	5	7	14	22	—	—	4	6	2	—	—	—	18	20
Heart	31	91	12	26	—	—	—	—	1	3	—	6	—	—	3	9
Liver	1	100	—	—	—	—	—	—	—	—	—	—	—	—	0	0
Spleen	1	100	—	—	—	—	—	—	—	—	—	—	—	—	0	0
Metabolic																
Pancreas	120	95	17	12	1	7	—	—	—	—	2	—	2	2	6	5
Thyroid	6	75	1	11	—	—	—	—	—	—	2	25	—	—	2	25
Blood	1	100	—	—	—	—	—	—	—	—	—	—	—	—	0	0
Chronic infections	20	83	1	11	2	8	1	1	1	4	—	—	—	—	1	17
Nervous and mental	12	93	5	10	—	—	—	—	—	—	3	7	—	—	3	7
Gastrointestinal	11	85	3	19	1	8	—	—	—	—	1	8	—	—	2	15
Metallic and food poisoning	3	100	3	50	—	—	—	—	—	—	—	—	—	—	0	0
Totals	375	878	71	15	25	6	1	—	11	3	9	2	3	—	52	122

mens from the disease types 1, 2, 5, and 6 of the Miloslavich classification, nor to attempt to differentiate between a cardiac and a renal dropsy by means of the micropolariscope.

As previously indicated our investigations have been conducted by means of Zeiss polariscope attachments, and without the use of the selenite plate. The micropolariscope examinations were carried out on the sediments remaining in the centrifuge tubes after the completion of the routine chemical and microscopic examinations. To assure ourselves of the validity of our technique as well as to familiarize ourselves with the morphology of the elements to be sought an intensive preliminary study of three months' duration was made during which period 225 specimens were examined. No record of coincidental findings nor diagnosis was kept. Thereafter a complete record of the chemical and microscopic findings was kept, and the diagnosis in each case obtained from the chart while the patient was still in the hospital. These latter were subsequently checked in doubtful instances against the patient's index file of the hospital giving the patient's final diagnosis on discharge.

Very early in our investigation there became apparent an inherent weakness of the micropolariscope examination which cannot be readily avoided. This arises from the presence in a large percentage of specimens of numerous crystals of comparatively no significance which are all highly anisotropic. It has been found possible to identify lipoidal elements morphologically among such crystals but where they are present in any number they flood the field of the microscope with crossed Nicols with light to such an extent that the identification of lipoids among them would be well nigh impossible and it became necessary to throw out such specimens and record the polariscope examination as "invalid." Twenty-two per cent of our examinations were invalidated in this way, bringing our total number of valid examinations down to 1146.

Another weakness of the polariscope procedure is suggested by our investigation rather than demonstrated. Approximately 95 per cent of the lipoidal positives observed have come from acid or neutral specimens. The question arises: Does the chemical complex resulting in the crystallization of a cholesterol ester in the human organism require an acid environment and may not these substances be present in urine in solution forms not detectable by the micropolariscope? The evidence would seem to indicate that it may. Of 20 specimens examined coming from diseases of the Miloslavich classification but one was alkaline, and this one did not show presence of any lipoids though all other seven of the eight examined from the same case were positive micropolariscopically for lipid granules or lipid casts or both.

Our observations do not throw much light on the physiologic chemistry of the processes by which the lipoids are produced and eliminated in the urine. One case of acute terminal nephritis of mixed type in a child is worthy of mention. The urine was positive only for albumin and blood for several days. Later, casts began to appear in increasing quantities. The micropolariscope examinations at this time were negative. The quantity and character of the casts changed very markedly over a period of several days, the number increasing and the casts becoming more and more coarsely

TABLE III
100 OBSTETRIC SPECIMENS

	NEGATIVE	PER CENT	INVALID	PER CENT	GRANULES	PER CENT	PER CRYSTALS	PER CENT	CASTS	PER CENT	EPITHE LIUM	PER CENT	PUS	PER CENT	TOTAL POSITIVES	PER CENT
Uncomplicated	24	100	2	9	—	—	—	—	—	—	—	—	—	—	0	0
Toxic	40	81	4	8	3	—	1	2	1	2	3	6	1	2	9	19
Surgical	20	95	—	—	—	—	—	—	—	—	1	5	—	—	1	5
Totals	84	89.4	6	6	3	3	1	1	1	1	4	4	1	1	10	106

TABLE IV
COINCIDENCE OF 152 LIPOID POSITIVE SPECIMENS

	GRAN ULES	PER CENT	CRYSTALS	PER CENT	CASTS	PER CENT	EPITHE LIUM	PER CENT	PUS	PER CENT	TOTAL POSI TIVES	PER CENT	PER POSITIVE OF TOTAL EXAMINED
Acid or neutral	49	32	12	8	18	12	61	40	5	3	145*	95	13
Alkaline	9	6	2	1	2	1	2	1	—	0	15	10	2
Chemical and microscopic negative	17	11	7	5	—	0	26	17	—	0	50	33	5
Albumin or casts	27	18	5	3	20	13	14	9	1	3	67	44	6
Albumin and pus	7	5	—	0	1	—	12	8	4	—	24	16	2
Pus	9	6	2	1	3	2	7	5	1	—	22	14	2
Sugar	3	2	2	1	1	—	5	3	1	—	12	8	1
Acetone postanesthetic	14	9	2	1	1	—	17	11	—	0	34	22	3
Acetone toxic	2	1	—	0	—	0	2	1	1	—	5	3	1
Red blood cells	14	9	1	—	—	0	3	2	—	0	18	12	2
Bile	—	0	1	—	2	1	2	1	—	0	5	3	1

*Includes 8 specimens containing more than one lipoidal element—5.3 per cent of the positives

granular in character. Waxy casts then appeared and for several days their proportion increased, the proportion of granular casts subsiding. The lipoids did not appear until three days after the first appearance of the waxy casts, fourteen days previous to the death of the child. The urine thereafter remained positive for the typical lipid granule for several days until no further specimens could be obtained, the patient developing a complete anuria.

It is our opinion that the lipid granule is a finding of graver significance than either the lipid epithelial cell or the lipid cast. It is reasonable to suppose that a small excess of lipid substances in the kidney might be eliminated in the protoplasm of epithelium and casts, whereas the chemical complex resulting in the production and elimination of the formed granule would be of a more serious nature. This hypothesis would seem to be borne out by the findings in the surgical group of specimens, under which the tumors, thyroid surgery, gynecology, and salpingectomy subgroups all show the highest percentage of the lipid positive in the form of lipid epithelium. The appendectomy, miscellaneous laparotomy, genitourinary, and minor surgery subgroups also show a high percentage of lipid epithelium compared to other lipid forms.

The most interesting finding in the surgical group is the fact that the small group of kidney surgery specimens was entirely negative for lipoids of any form, while the gall bladder surgery group showed a very high percentage of specimens positive for the granule. The total positive finding of 27 per cent for this group represents in fact the closest approach of any group to the maximum finding of 29 per cent in the degenerative diseases of the kidney, a matter which we consider of some significance. Coupled with the experimental fact quoted by Miloslavich to the effect that hypercholesterolemia of itself does not result in the elimination of lipoids in the urine until after the kidney has been artificially damaged by the injection of a poison, it would seem to postulate the presence in the kidney in these degenerative diseases of some substance derived from source or sources exterior to the kidney itself, which substance can be caused to appear in the kidney by surgical trauma of the gall bladder.

The positive findings in plastic surgery (herniotomys) also were high. It is to be regretted that the number of specimens available from these two groups was not sufficient to carry greater weight.

The findings in the group of specimens from medical cases serve only to confirm Miloslavich's assertions with regard to the importance of urinary lipoids in degenerative diseases of the kidneys, the incidence in this subgroup being 29 per cent. A high incidence of 25 per cent was also found in diseases of the thyroid, the finding consisting, however, of but two specimens containing lipid epithelium out of a total of eight examined.

The obstetric group findings serve only to substantiate the fact that in normal pregnancy no injury to the kidney is to be expected.

We have already emphasized the fact brought out by Table IV of coincidence of 152 positive specimens that 95 per cent of these have been found in acid or neutral urines. This table shows that the micropolariscope examination may be expected to disclose positive findings in 33 per cent of

total lipid positives or 5 per cent of all pathologic specimens where all other findings are negative. This percentage of total specimens is comparatively small, and in view of the high probability of the examination being invalidated we cannot agree with Miloslavich that the micropolariscope should be employed as a routine procedure except in certain selected groups of cases from which further information is to be desired. The table also shows that lipoids detectable by micropolariscope may be found in conjunction with all of the other substances of a positive nature commonly looked for in urine, being associated in greatest frequency with albumin and casts.

SUMMARY

One thousand, one hundred and forty-six valid routine micropolariscope examinations of pathologic urine have been completed and the results have been tabulated. A general discussion of the incidence of lipoids in urine and their diagnostic significance has been given. A tool has been used which has disclosed its own weakness, but has brought forth information which demands to be checked by other means.

CONCLUSIONS

1 The incidence of lipoidal substances detectable by the micropolariscope in pathologic urine is approximately 13.3 per cent, the frequency of their occurrence being comparable to that of the finding of casts, and the weight of their diagnostic significance being comparable if not greater.

2 It seems probable that the micropolariscope examination is invalid in a large proportion of specimens and that the true incidence of the lipoids may be much higher than indicated.

3 Evidence is offered suggesting the possibility that conditions of the gall bladder of a pathologic nature, not at all clearly understood, may bear an etiologic relationship to the degenerative diseases of the kidney, and further investigations of the occurrence of lipoids in urine from cases of gall bladder as well as hemia surgery are to be desired.

4 The physicians should make an urgent demand of the biologic chemists for a simple chemical test for the detection of lipoids in urine. All that we in the routine clinical laboratory can do is to record and tabulate and report what we have observed.

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MERCURY POISONING, ITS CLINICAL DIFFICULTIES AND ITS PATHOGENESIS

REPORT OF TWO CASES OF BROWN SUBIMATE KIDNEY*

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ACUTE mercurial poisoning is a familiar and well defined syndrome, with characteristic history, symptomatology, laboratory findings and pathologic picture. But not always is a history available. The poisoning may be accidental or homicidal and neither the victim nor attendants ever suspect the correct cause. It may be suicidal and the patient carefully withhold the information which would institute life saving remedies. As a frequent penalty of ill advised therapeutics, or of attempts at contraception or abortion mercury poisoning is admitted often only with faint hearted reluctance or not at all.

Lacking a history one may be led to the correct diagnosis by the typical clinical picture of the acute cases. But the direct and differential diagnosis is not always as simple as textbooks outline. The cases are rare. Unless one is unusually alert for it, without a history mercury may never even be considered. When it is its consideration may be submerged by more common syndromes which closely simulate it.

Laboratory methods fail us usually because we do not even think to use them. Cases are often unwittingly stumbled upon during routine chemical examinations. Nor does every finding of a trace of mercury mean mercury poisoning. Industrial or accidental exposure to minute quantities, even amalgam fillings may cause the appearance of small amounts of mercury in the excreta without any serious symptoms being attributable to it.¹ An adequate amount of mercury must be found to incriminate it as the etiologic factor.

Even the pathologic picture is subject to puzzling variation. Mercury works its fatal way in devious manners. It may rapidly cause death by direct cardiomuscular damage with circulatory failure and high anhydremic leucocytosis before there has been time for appreciable kidney damage.² Death may result from slower but direct mercurial action on all the organs, from the incidental enteritis or other infections or starvation or dehydration acidosis, or from an accidental infection without sufficient renal damage ever appearing. Massive renal damage with true uremia is however the lethal factor in typical cases.

The histologic picture depends on the dose, the interval after which death occurs, the associated pathologic changes and other less well under

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stood factors. But the time sequence of changes has been very definitely worked out.³ With a given fatal dose, and a known time after ingestion, and the associated changes determined, the microscopic picture may be more or less accurately anticipated. Thus mercury offers several varieties of renal picture, yet each is definitely established. These changes are not absolutely specific, for close resemblances occur with other poisons, such as bismuth, uranium, chromium, B-naphthol, sulphuric acid, oxalic acid, and hydrochloric acid. But they are sufficiently characteristic so that, with the slightest circumstantial support, they deservedly claim the name of "mercury kidney."

As a diagnostic aid, the microscope has therefore proved of great value, particularly when the diagnosis has slipped through the preceding elements in diagnosis. In two cases at the Cook County Hospital, in which a history of mercury was never obtained, and the clinical picture evaded diagnosis, microscopic examination led to confirmatory chemical tests which established it at least postmortem. Both recalled attention to several interesting details in renal pathology. Neither presented the usual picture associated with mercurialism. In each, the obscure clinical picture, reanalyzed, was secondarily clarified.

CASE 1—

Clinical History—A white girl, seventeen years old, entered the hospital with an Admitting Room diagnosis of pelvic peritonitis. She stated that she had felt quite well up to four days before, when at midnight she was suddenly seized, for no apparent reason, with a severe, sharp pain. This pain began in the left pelvic region and radiated to the right side of the abdomen. She vomited several times, and once on the first day vomited blood. There was no desire for food, swallowing gave her pain, and there was pain in the epigastrium. She stopped urinating about sixteen hours after the onset of this attack. The nausea, the abdominal pain, and the vomiting persisted. She could take nothing at all by mouth. The pains grew worse.

The past history only added confusion. The patient had had "sleeping sickness" four years previously, and since then many fainting and dizzy spells. The mother volunteered that she had really been ill on and off for three years with vague abdominal pains for which an appendectomy had been fruitlessly done. She stated also that the patient had often had such urinary disturbances, and on a few occasions could not urinate for three or four days. She insisted that the present condition had all come on after drinking some ginger ale, of which she herself had partaken without harm.

The patient's menstrual history was normal. There had been a slight leucorrhoea for a year, but no pregnancies or abortions. She firmly denied any medication or poisoning.

Physical Examination—She was fairly well nourished. Her blood pressure was normal, 120/75, her pulse rate 90. Her breath was foul. Lips, teeth, tongue, and pharynx were dry and coated.

Heart and lung findings were altogether negative, and there was no edema.

The abdomen and genitalia were very tender, wherever touched. The hymen was deflorated. Vaginal examination was negative.

Catheterization of the bladder yielded only a few cubic centimeters of dark, bloody urine with a few epithelial cells. Cystoscopy was negative.

She suffered a rather severe diarrhoea with loose, black stools which gave a four plus reaction for blood.

Laboratory Examination—The leucocyte count was 11,500 with 85 per cent polymorpho-nuclears. Temperature and respiration were normal. The blood Wassermann was negative. On the day of entrance, the urea nitrogen was 120 mg per 100 cc, creatinin 12.4, cholesterol 200 and carbon dioxide combining power 46 volume per cent.

Clinical Diagnosis—Urinary suppression, the etiology of which could not even be ventured

Further Course—Treatment of the anuria was pushed desperately. Hot packs were applied to the lumbar region. She was given daily hypodermoclysis of 3000 cc of normal saline solution and 1000 to 1500 cc of 10 per cent glucose intravenously with insulin. Sweating was tried, gastric lavage was done. Morphine was given to quiet her delirium and restlessness.

The second day after entrance a decapsulation of the left kidney was performed. The stupor, the vomiting, the diarrhea, the anuria persisted. Her pulse became weak and thready. Digitoxin and pituitrin were regularly given. Continuous proctoclysis was instituted. Edema never appeared though she received a tremendous quantity of fluids. The blood pressure rose to 160/80.

The blood chemistry remained about the same despite all treatment. On the tenth day of her illness she passed 160 cc of turbid urine containing many red blood cells. On the eleventh day she passed 210 cc of similar urine, but on this day lapsed into deeper coma and died.

Postmortem Examination—The lips were crusty, the teeth were in fair condition. In the left lumbar region was a recent surgical wound 15 cm long. Though subcutaneous edema was absent there was a bilateral hydrothorax of 1000 cc of clear fluid in each plural cavity. Hydropericardium and ascites of 500 cc and marked edema of the lungs, liver, and brain. There were striae albae in the lower abdomen and an ancient appendectomy scar.

The heart was of normal weight 300 gm but above the valves was the longitudinal wrinkling of a syphilitic aortitis. In the urinary bladder was 300 cc of yellow cloudy urine which gave a positive albumin reaction. There were punctiform hemorrhages in the mucosa. The uterus was hypoplastic only 4 cm long. Its mucosa was covered by soft blood clots. The left ovary contained a corpus hemorrhagicum 10 mm in diameter.

The stomach and small intestine were but little changed. The gastric mucosa was but slightly congested, the ileal mucosa pale. The cecal mucosa however was deeply injected, dark red grey with several transverse superficial ulcerations. These ulcers had indented edges, a light yellow grey base and measured from 1 to 3 cm in diameter.

The right kidney alone weighed 300 gm, was soft and wet. The capsule stripped readily leaving a smooth, pale grey red surface studded by a few minute deep red areas. Surfaces made by section showed the cortex 10 to 15 mm thick, pale grey red with obscure markings. The medulla was darker red. The left kidney weighed 320 gm. The capsule had been previously removed and on the convex margin were two longitudinal incisions extending into the kidney substance for a distance of 1 cm. On the anterior aspect near the hilus there were two irregular deep red areas with pale grey centers 25 and 15 mm in diameter extending 10 mm into the parenchyma.

Kidney—There was very marked dilatation of the convoluted tubules. The epithelium was very low. In some of the tubules it was flat to moderately high cuboidal with oxyphilic granular cytoplasm. Some of these cells were vacuolated. The nuclei were normal in number, and varied from large vesicular to small pyknotic ones. Most of the tubules however were completely relined by flat to low cuboidal cells with basophilic homogeneous cytoplasm, indistinct cell membranes and very numerous large nuclei rich in chromatin which bulged into the lumen. An occasional mitotic figure was seen among these nuclei. The lumen of the tubules contained fine branched threads of a pale stained material and single desquamated vacuolated cells. Some of the tubules were filled by fibrinoid material, others contained homogeneous hyaline material and a few showed erythrocytes. No calcium concretions were to be seen.

The Bowman's spaces of the glomeruli were wide and empty. The parietal epithelium of some was swollen, with numerous proliferated nuclei. Many of the capillary tufts were narrow, with the intraglomerular portion of the afferent vessels distended but empty. The interstitial tissue was diffusely increased, loose strikingly edematous. There were occasional single lymphocytes and plasma cells but no accumulations thereof. The medulla was markedly hyperemic.

The whitish and dark red areas away from the surgical incision in the left kidney showed a diffuse necrosis of the renal parenchyma, with extensive recent extravasations of blood. Here and there, the necrotic tissue was infiltrated with polymorphonuclear leucocytes. The arteries in the necrotic area were partly filled by fibrin. Specimens were then submitted to the coroner's chemist, Dr R. W. Webster.

Chemical Examination—

0.00541 gm of mercury (calculated as bichloride of mercury) per 140 gm of kidney tissue

0.00157 gm of mercury per 290 gm of liver tissue

0.0005 gm of mercury per 219 gm of heart tissue

0.00015 gm of mercury per 213 gm of uterus tissue

Anatomical Diagnosis was essentially

Mercury bichloride nephrosis (atypical form), with ulceropseudomembranous typhilitis

CASE 2—

Clinical History—A white woman, twenty-eight years old, was admitted to the gynecologic service with a diagnosis of bilateral salpingitis. Nine years previously she had given birth to twins, and one year later to a third child. For eight years her menstrual periods were uninterrupted, and symptomless except for a thick white discharge and slight premenstrual cramps. The menses were always regular until July 21, 1929, when they stopped abruptly.

She missed the August period. Shortly after this, about September first for no reason at all she insisted, she began to have pains in both lower quadrants, especially the right, as well as pain in other parts of the abdomen and over the sternum. After a few days, there began spells of vomiting. She would vomit seven or eight times a day. This persisted, so that for four or five weeks she was unable to hold any food down at all. In the last week before entrance she was unable to retain even water.

During these five weeks she salivated excessively so that she was always expectorating or wiping her mouth. She suffered from terrific thirst, and wanted to drink all the time. She was constipated, but noticed black, tarry stools at times. Since the beginning of her illness there had been a marked oliguria. She wondered that she had to urinate only once during the day, and only once at night. A few times blood was seen in the urine.

Physical Examination—She appeared to be acutely ill. She was quite obese, had a blood pressure of 118/70, a very rapid pulse of 160, an occasional chill with a temperature which never exceeded 100° F and was usually normal. The face was flushed. A distinct jaundice was present. Heart and lung findings were negative. The area of liver dullness appeared to be decreased.

On vaginal examination, both adnexa were tender but contained no masses. The vaginal mucosa showed the deep purple of Chadwick's sign, and Hegar's sign was also positive. The uterus was soft, and enlarged to the size of a two and one half months' pregnancy. The cervix was eroded, with many nabothian cysts.

A catheterized urine specimen was bloody and opaque. The white blood cell count was 12,350/mm³; the erythrocyte count was 4,900,000, the hemoglobin 90 per cent. The Wassermann reaction was negative.

Clinical Diagnosis was tentatively

1. Pregnancy with pernicious vomiting
2. Pelvic peritonitis, and
3. Endocervicitis

In attempted treatment of the pernicious vomiting, all oral intake was stopped, hypodermoclysis and proctoclysis were instituted. But the vomiting continued, and the patient was transferred to the obstetric service in contemplation of more radical therapy.

Further Studies—A blood chemistry report was returned with a urea nitrogen of 75.5 mg/100 cc of blood, a creatinin of 2.2, a cholesterol of 178, and an icterus index of 30. A phenolsulphonephthalein test returned less than 15 per cent of the dye in three hours. In

the Fishberg test, only 200 of the 1000 cc of water given were excreted within four and one half hours. The highest specific gravity of the urine was 1.016. It still contained albumin and casts, bile blood and white blood cells.

Ophthalmoscopic examination showed numerous petechial hemorrhages in both fundi. She was evidently quite toxic. Her lips were dry and parched, with beginning sordes. Her gums were injected and there was a foul odor to her breath. Yellowish white membranes were seen over her injected pharynx. Both lumbar regions were tender.

Clinical Diagnosis was then

1. Pregnancy with toxemia.

2. Acute nephritis or acute yellow atrophy of the liver to be considered.

The patient improved somewhat for a time and a cystoscopy was done. Its findings were negative. Even the phenolsulphonephthalein appeared from each ureter in normal time but the percentage returned was very low. Then she began to complain of epigastric distress. Her vomiting persisted despite all treatment. Under spinal anesthesia a therapeutic abortion was done.

There was still not the slightest suspicion of poisoning. The husband then admitted that just before the onset of her illness the patient had taken quinine and ergot pills to help the lower abdominal pains restore her menses. The mother added that she had also taken some Gold Medal pills and that these had precipitated the vomiting.

There was no improvement in her general condition. Her azotemia rose to a urea nitrogen of 175, a creatinin of 16. A serosanguinous discharge issued from the vagina. All supportive measures failed and she died about two months after the onset of her illness, five days after the therapeutic abortion. After three weeks of hospital observation the diagnosis was still undetermined.

Postmortem Examination—There was a slight diffuse icterus. The teeth were in good condition. Colostrum was expressible from both breasts. The ankles were slightly edematous. The heart was of normal weight, 255 gm, but the myocardium was softened by a severe parenchymatous degeneration. The spleen and liver were similarly softened by cloudy swelling. The liver was 1920 gm in weight. A thick, tarry black bile filled the gall bladder. The intestines were grossly normal. The brain substance was edematous, the leptomeninge pale.

The uterus was enlarged to four fingers above the symphysis. It was 12 cm long, 9 cm wide and 5½ cm anteroposteriorly. Its wall was 18 mm thick, pale greyish brown and very soft. The cavity uteri was lined by soft, adherent, light pink grey to yellowish grey material. In the lower uterine segment and cervix there was a light yellow soft membrane firmly adherent to the wall. The right ovary contained a 10 mm corpus luteum.

The kidneys together weighed 530 gm. Their consistency was diminished. The capsula stripped readily leaving a light brown smooth surface. The cortex was 9 mm thick, its markings obscure. The medulla was purplish grey.

Microscopic Examination—*Kidneys*. This furnished the first clue to the correct diagnosis. The convoluted tubules were seen to be markedly dilated. Many of them were lined by low epithelium which showed no intercellular membranes and was markedly basophilic. Their nuclei were very numerous, lay close together and often bulged into the lumen. In places small fat droplets could be seen. These basophilic homogeneous multinucleated tubules stood out sharply among the other tubuli contorti with their higher, finely granular, more oxyphilic cytoplasm and fewer and paler nuclei. The lumen of the latter tubules was filled by granular vacuolar debris.

In the glomeruli distinct swelling and nuclear proliferation of the parietal epithelium was readily observed. The Bowman's space often contained a vacuolar material similar to that seen in the convoluted tubules. Many of the connecting tubules contained clumps of pale erythrocytes or of erythrocytic debris. There was a very occasional connecting tubule which contained a small spherical calcium concretion. The interstitial tissue was somewhat increased, loose, edematous. It had a few histocytes containing fine lipid granules but no cellular infiltrations.

These contain ferrous sulphate, aloes and oil pennyroyal with no trace of mercury.

Liver The centro acinar liver cells and Kupffer cells contained yellowish brown pigment granules

Uterus There was diffuse necrosis of the internal third of the wall, separated from the rest by a distinct zone of demarcation with numerous degenerated leucocytes. There were clumps of cocci in the necrotic area. Beyond the pyogenic wall, some of the lymph vessels were transformed into intramural abscesses.

Specimens were then submitted to the coroner's chemist.

Chemical Examination—0.0044 gm of mercury in 2.51 gm of kidney tissue. No mercury was found in the liver.

Anatomical Diagnosis was essentially

- 1 Subacute mercury bichloride poisoning
- 2 Diphtheritic suppurative endocervicitis
- 3 Postpuerperal suppurative endometritis

Clinical Discussion—The first case, an example of acute mercury poisoning, the second an example of subacute mercury poisoning escaped clinical diagnosis primarily for lack of history. The careful silence maintained by both patients with respect to poisoning proved an effective shield. Mercury was not suspected even enough to call for chemical tests antemortem.

That mercury was absorbed is certain, but how it was introduced is altogether obscure. Poisonings have been described by every possible route and orifice. Oral ingestion is the most common, but in neither of these cases was there sufficient local corrosion to establish or to even call attention to an oral route.

A particularly potent source of fatal and readily disguised mercury poisoning is the vaginal route. Numerous cases have been described. The mercury is applied usually as a contraceptive, or as an abortifacient or as more discreetly called an "emmenagogue." Even as a disinfectant douche, it may be rapidly absorbed with serious result. Le Doux,⁵ Rynd⁶ and others have therefore decried its free, even therapeutic prescription. Absorption from the vagina is rapid because of its rich lymphatic supply and acid reaction.⁷ It is dangerously increased by the local hyperemia of hot douches in which it is used, by any local inflammation, by the irritation attending attempts at abortion, even by the hyperemia of a normal pregnancy.⁸ For example, as a douche in chronic endocervicitis mercury may long be harmlessly used until the vaginal hyperemia of a supervening pregnancy causes toxic absorption. The vaginal introduction of mercury under these conditions bears an especially grave prognosis. It should be guarded against and watched for.

The average fatal dose, as given by Goldblatt,⁹ is almost 2 gm (28 grams), but less will suffice. The severity of symptoms is not proportional to the dose, because absorption and individual susceptibility are important variables.¹

In retrospect, the symptomatology of both cases seems typical of an acute and of a subacute mercury poisoning. But no such syndrome was suspected before autopsy revealed the diagnosis.

FOUR STAGES

Landau and Fejgin¹⁰ have outlined the clinical history into four typical stages with definite time sequence.

The first stage of local corrosion lasts from intake of the poison to the beginning of anuria, usually within twenty four hours. There is severe local pain and burning, and other symptoms which vary with the route of introduction. In oral cases an early preliminary period of vomiting soon appears (within fifteen minutes to several hours). The sooner it begins after ingestion, the better is the prognosis.⁹ In extraoral cases it is absent.

The corrosion symptoms subside in the second stage of anuria which lasts from the second to the seventh day or longer. Anuria lasting more than seven days, as it did in the first case, is uniformly fatal. The anuria is attributed to the loss of water by the vomiting and diarrhea which supervene, to the low blood pressure, and to the stuffing of renal tubules by desquamated tubular epithelium. Elwyn¹¹ ascribes it to constriction of the renal vessels by the tensed capsule of the swollen kidneys. This would support the rationale of therapeutic decapsulation. But Held¹² has indicated that those cases which appear brilliantly benefited by decapsulation are operated upon about the time when spontaneous recovery usually appears anyway.

Edema is absent, probably largely from lack of water. When it is supplied, effusions may appear. A marked azotemia rapidly develops. This is the result of two factors, the paralysis of kidney function which stops excretion, and the severe toxic increased destruction of proteins by mercury all over the body.¹³ In exceptional cases, the blood pressure is raised, usually it remains low. The retained products of metabolism themselves aggravate the renal and general damage.

If the patient lives to urinate again, the danger is not yet over. The stage of oliguria may go on even to a polyuria. With polyuria there may be a steady decrease of the blood nitrogen retention as the patient passes through the fourth stage of recovery. But the oliguria may proceed to a fourth stage of increasing intoxication and death, with or without polyuria. For the concentrating ability of the kidneys may be so badly damaged that the hyposthenuric urine excreted is almost plain water. The tissue protein destruction continues in the third stage beyond the power of the crippled kidneys to cope with.

Corrosion gives the first symptoms, toxemia the second, and uremia closes the picture.

What factors will determine the return of kidney function and the cessation of tissue destruction, or otherwise, after a period of anuria is the most important problem in therapy. Very often the return of function is prevented and "erweisszerfall" continues, not by reason of the original mercurial onslaught, but by previous renal damage, circulatory weakness, starvation and dehydration, exhaustion, by operative or medical insults, or by supervening infections to which these patients are especially susceptible. Thus the histologic picture of the cases described, particularly the second, indicated that recovery might have taken place if extrarenal factors including the operative procedures and the endometritis had not turned the scales. Supportive measures should therefore be the keynote of therapy.

Chemical examination offers the single most reliable antemortem diagnosis. Mercury can be detected in the urine, feces, gastric content or blood.

with great facility.^{14 1} If only mercury is suspected, it is a simple matter to confirm or disprove it by a Reinsch test.

PATHOLOGIC DISCUSSION

Pathologic examination has often retrieved the diagnosis. Askana¹⁶ defined the kidney changes by their gross appearance into three stages:

- 1 Red primary stage
- 2 Grey-white sublimate kidney
- 3 Red sublimate kidney

First Stage—The red primary stage stretches through the first twenty-four hours after absorption of the sublimate, the period of local corrosion. It is only exceptionally seen at the autopsy table. The kidneys are red because of a toxic hyperemia which dilates all the capillaries of cortex and medulla. It begins with extreme rapidity within five minutes after absorption.⁴ The first parenchymal change is an edema and hyalinization of the glomerular tufts and a thickening of the reticular substance which may be followed shortly by necrosis of the endothelial cells.^{1 b} The glomerular damage reaches its height within six hours but then quickly subsides.¹⁷ Only traces of this glomerulo-nephrosis could be seen in the sections first described, in the swelling and proliferation of the Bowman's capsular epithelium.

Tubular injury begins almost as quickly but continues to progress in severity. The site of election for the worst changes is the convoluted tubules of the third order, and its transitional part with the descending limb of Henle's loops. The damage starts here and spreads in both directions.¹⁸ Necrosis is soon seen here, but in the rest of the tubule only granular swelling and fatty or hyalin changes are all that is usually found.

It is only because of their precarious role as excretory organs that the kidneys are so severely implicated. If one ureter is previously ligated, the corresponding nonfunctioning kidney suffers not the slightest damage from mercury absorption. Whether the mercury acts directly on the epithelium¹¹ or destroys it only secondarily by the anemia following a direct vascular spasm (Kaufmann) or a neurovascular irritation (Ricker), is in question. Stracke¹⁹ experimentally made direct binocular observations of such vascular disturbances. The marked idiosyncrasies which exist toward mercury speak more for its action on a sensitive neurovascular apparatus than on stable parenchymal cells.²¹ On the other hand, Kosugi denies the appearance of such vascular changes. The extreme rapidity with which necrosis appears, the diffuse involvement of all tubules, the selection of a specific part of each tubule argue more for a direct cellular damage by mercury than for a vascular mechanism. Be its mechanism what it may the mercury necrosis pushes the kidney rapidly on into the second stage.

Second Stage—The second stage of the grey-white kidney is mercury necrosis in full bloom. It is reached within twenty-four hours and lasts for seven days or slightly longer, through the clinical period of anuria. It gives the usual picture illustrated in textbooks. The kidneys are swollen, pale grey or whitish grey. The striking pallor is the result of a diffuse oligemia

caused not by contraction or occlusion of the vessels but by their compression by the swollen tubular epithelium. A coagulation necrosis strikes the transitional part of most of the proximal convoluted tubules. Only a few are spared. The cells become small, are sharply outlined, with a homogeneous cytoplasm and pyknotic nuclei. Later, hyaline necrosis also appears giving swollen, granular, acidophilic cells with large vesicular nuclei. This is ascribed to acidosis or other extramercurial toxins for it may appear alone, even after all trace of mercury is gone and prolong the damage.²

The necrotic epithelium of the convoluted tubules then separates from the basement membrane, and stuffs the lumen. This desquamation depends largely, according to Heineke,¹⁹ upon actively regenerating cells from the basal epithelium. The young cells grow under the dead ones and push them out. They follow them into the lumen, surround the sequestered cells like an involucrium and grow in between them. They themselves may be caught at times in the coagulation or hyaline necrosis (see Gorke¹ and MacVider²). Many agree with Heineke that the young cells take active part in the resorption of the necrotic ones. Fahr³ contests this view holding that the regenerating cells do not digest the old ones, but only passively grow in, wherever they find room.

Regeneration—The extraordinary rapidity and activity of these regenerative processes is the most specific characteristic of the sublimatic kidney. It is the single and most constant diagnostic feature in this and later stages. Regeneration begins very early from the undamaged and even from the slightly damaged cells. Within seven days from ingestion of the poison the necrotic cells may be desquamated and the tubules completely relined by a new growth.

The new lining is readily distinguishable from the old. The cells at first are flat. They are poorly separated from one another so that they form almost a syncytial ring. They are crowded strikingly with numerous deeply chromatinized nuclei which bulge into the lumen from the flat cells. The nuclei are so numerous that they seem to lie one on top of the other around the lumen. The glomerular capsular epithelium is similarly affected. The cytoplasm of the young cells is basophilic and homogeneous, light blue in sharp contrast to the purplish red of the mature or the light red of the necrotic cells. As the lining matures it grows taller and more regular, cell membranes become distinct, cytoplasm turns granular and oxyphilic nuclei become less numerous. The young cells function poorly. As they mature, function returns.

Cast of necrotic cells still lie within the lumen of the tubules. Clearing the lumen by resorption of these casts, and pushing the epithelial relining to completion is the feature of the third stage.

Third Stage—The stage of the red sublimatic kidney begins usually after seven days, after the period of anuria. The redness is a result of a new hyperemia which is imposed upon the kidneys by two factors. One is the hyperemia of active regeneration. This almost always makes its appearance, but is moderate and lends only a light brownish color to the kidneys. In both of the cases described in this paper only this color was reached. The other

factor is more variable. It is a hyperemia which is intimately concerned with calcification. It may be absent, as it was in these two cases, but when present offers to the kidneys the typical red color of the third stage.

Calcification—Calcification of the necrotized cells first appears in the third stage. It affects only the cells involved in coagulation necrosis, not those involved in the hyaline necrosis. It affects particularly the cells disintegrating slowly in coagulation necrosis. A certain "vita minima" is necessary to induce the settling out of calcium, which completely destroyed cells cannot muster (Gorke²⁴). Calcification, however, is not specific to the mercury kidney. It occurs to some extent in copper, iodine and phosphorous poisoning, in simple anemic infarcts, in nephritis, in nephrosclerosis, even in extrarenal disturbances. It may be slight indeed or absent altogether in proved mercury poisoning. Thus it is always absent until the third stage (about one week), and disappears as soon as the coagulation necrosis debris is cleared out. It is unlikely to appear where there is rapid necrosis, in some cases it never does appear.

Schmidt²⁶ has suggested as a likely factor in determining the presence or absence of calcification, the severity of intestinal tract damage. Calcium is normally excreted by the intestinal tract. If its mucosa is destroyed by mercury, the calcium is unloaded through the kidney. He contends therefore that calcification is seen in the kidneys particularly in those cases of mercury poisoning where the intestines are extensively involved. This is generally agreed. In argument he reports calcium concretions in the kidney in ordinary cases of dysentery. Only Lemke²¹ dissents in reporting a case of mercury poisoning without colitis, without calcemia but with marked calcium deposit in both kidneys. Colitis is probably only one of the several factors involved. The first case described in this paper showed a marked colitis but no calcification at all, the second showed no colitis at all but a slight calcification.

A marked hyperemia attends the calcification of the necrotic cells. It brings in calcium with sufficient speed to enable it to accumulate about the dead cells. In turn the calcification arouses an active inflammatory response in attempts to evacuate it. The hyperemia increases and gives to the kidney of the third stage its typical deep red color. Leucocytes infiltrate the edematous interstitial tissue and even wander through the walls of the cast-stuffed tubules. They penetrate the masses of calcified necrotic cells and effect their resorption. The casts become smaller, are resorbed entirely or swept away, and the lumen is cleared. Simultaneously, the new epithelial lining matures and function is restored.

Slight calcification may be cleared up without an inflammatory reaction, with no interstitial infiltration and with only the hyperemia of active regeneration to give these third stage kidneys a light brown color. When however because of enterocolitis, calcemia, or for other reasons mentioned, any appreciable calcification occurs, there is marked interstitial infiltration and a severe hyperemia to give the more typical red sublimate kidney of this stage. Observations by Takahashi,²⁷ Schneck,²⁸ Hunter²⁹ and many others attest this fact. Vollhard³⁰ thus distinguishes two types of mercury nephrosis

in the third stage, one without inflammatory reaction, and the more usual one with inflammatory reaction. Both of our cases fell in the first and more infrequent category with little or no calcification, no interstitial infiltration and only light brown sublimate kidneys, instead of the usual red ones.

CONCLUSIONS

Two cases of fatal mercury poisoning are described which clinically were obscure. Lack of history was the chief diagnostic handicap. It led readily to incorrect interpretation of clinical symptomatology which in retrospect was typical for an acute and for a subacute poisoning. So remote was the suspicion of mercury that chemical examination was not even tried.

Histologic examination retrieved the diagnosis in both cases. It demonstrated the extreme activity and rapidity of regenerative changes in mercury nephrosis. Thus lively regeneration is more specific for mercurialism than is calcification which indeed was slight or absent in both cases. The pathogenesis of mercury nephrosis is reviewed. Attention is called to the fact that when calcification fails to appear, inflammatory reaction in the kidneys is likewise absent. There are then no interstitial infiltration and insufficient hyperemia to produce the typical red sublimate kidney of the third stage. Both cases described lacked this inflammatory reaction and are presented as examples of the less frequent brown sublimate kidney.

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DETERMINATIVE STUDIES OF AN UNDESCRIBED SPECIES OF GRAM NEGATIVE CORYNEBACTERIUM (C. QUASISTERILUM)*

BY GEORGE WILLIAM COOPER,† B S, A M, M D, WASHINGTON, D C

THE sanitary engineer or surgeon may at times be confronted by the appearance of irregular and bizarre species in his bacteriologic studies of the water he is investigating or using. An orange chromogenic gram negative bacillus was isolated from the sterile tap water system of the Urologic Clinic on April 18, 1929. Five subsequent attempts at short intervals to isolate this or other organisms gave negative results upon plain agar and dextrose peptone broth infusion. The organism was first isolated from the water of cystoscopic examining rooms four and five. On June 19, 1929, the cultures from the sterile water in rooms three, four and five were again positive for a gram negative chromogenic bacillus. It developed that the growth potential of the organism was cyclic, the negative phase varying from six to eight weeks following the flushing of the sterile water system with live steam. The activity of the organism is revealed in the following studies and does not confirm the assumption of propagation by spores.

The cystoscopist is likely to encounter such organisms in the use of certain supposedly sterile waters. This appears especially true when stock distilled waters are used and taken directly from large supply basins, even though the latter are seemingly faultless. The elaborate distilling and storage plants for sterile water tap systems are not beyond some critical comment and sterilization of water immediately before being used for cystoscopic examination and surgical procedures would appear to be ideal.

The pathogenicity studies of the organism here reported were consistently negative. Mice, guinea pigs and rabbits were used. This report is recorded as a guide to surgeons who may find this organism in their distilled water. They may consider it a nonpathogen in the usual sense but the question arises as to the possibility of implantation and growth upon an already diseased tissue.

The organism is an orange, chromogenic, aerophilic, mesophilic, non-motile, gram negative bacillus. Its normal habitat is water. The bacillus is medium sized, as a rule 0.5 by 3.0 μ but many individuals are quite small and "diplococcoid" in appearance. Diplo forms are very common while chain formation is fairly frequent in certain cultures. Filamentous forms are found in old cultures which also show false branching. Wadsworth and the India ink methods show no capsules. Flagella stains are negative. Moller's and Ziehl-Neelsen stains show unterminal granules usually subterminal but oc-

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asionally terminal. Spores are not observed. There is apparent swelling of the cell bodies at the granules. Longer forms, 5 microns, occur and may show central or excentric granules. The granules are metachromatic by Neisser's staining. Rarely a chain of granules occurs throughout the length of the longer forms.

The moist-heat resistance test confirms the absence of spores by the various spore staining methods.

Extensive pathogenicity studies on mice, rabbits and guinea pigs give no consistent or conclusive reaction.

Cultural reactions are quite distinctive. The colonies tend to remain discrete, even on ascitic agar, upon which they grow more profusely than on plain agar. On the latter the colonies are usually larger but much less numerous than those resulting from a similar technic of inoculation upon ascitic agar. The colonies on plain agar are round or slightly oval with smooth edges and with a distinctly raised, well demarcated and more deeply pigmented central zone which is brilliant orange in color at ninety-six hours. The lighter color of the outer zone of the colonies is seen to be a bright yellow. The pigment is also well developed, as a rule, at seventy-two hours. The raised central zone occupies just about half of the total surface area of each colony.

The optimum temperature on plain agar medium is 37° C. The organism is relatively a slow grower, even on ascitic and reinforced media. It is even more retarded on plain agar at 25° C.

Acid formation is slow and occurs only on maltose, saccharose, raffinose, arabinose, and the hexoses. On dextrose broth there was one-plus acid, three-plus pellicle and four-plus sediment at seven days of growth. At ten days the acid and growth appearance was approximately as at seven days, augmentation of either being difficult to make out. Other dextrose, galactose, and sucrose cultures showed acid formation varying, of course, with inoculation dosage. Thus there was often a faint trace of acid present at seventy-two hours, a heavy trace at ninety six hours, a 1-plus acidity at seven days, 2-plus at two weeks, and 4-plus at four weeks. Pellicle formation occurred on liquid sugars, alcohols, and glucosides but was uniformly retarded. No gas was found on any of the fluid or solid media. Litmus milk was unchanged at five days.

Growth is more rapid and profuse on whole blood agar and potato than on plain agar. The gentian-violet potato colonies at one hundred and forty-four hours are larger than those on plain potato but are always less numerous, the dye restricting the growth of some individuals, the colonies measuring 4.0 to 7.0 mm. at one hundred and forty-four hours on gentian-violet potato, high and bulging above the surface of the medium. They are round or oval, moist and dirty yellowish in color.

lauer tests were negative. Two methods were used under parallel test conditions to check the reduction of nitrates: (1) the test as advised by S. A. B. in "Standard Methods" and (2) the nitrous acid starch iodide test. These tests were uniformly negative and there was no apparent formation of ammonia. The organism does not reduce urea and does not utilize citrate agar in its metabolism.

The bacillus, classified after the 'Key for the Identification of Organisms of the Class Schizomycetes' of the Society of American Bacteriologists (1923) is found to be of the order 'Actinomycetales', family 'Mycobacteriaceae', genus 'Corinebacterium'. No tribe category is specified by the Bergey *Determinative Manual* under the *Mycobacteriaceae*.

The character distribution of the organism roughly parallels that of certain of the *Bacillaceae* in so far as the fragmentary known 'characters' of some of the latter are recorded, but it does not form spores and in most respects resembles the *Mycobacteriaceae*. The organism likewise resembles a species of *Flavobacterium* in many respects (*Flavobacterium oxalis*—Wright).

The bacillus under consideration, then, cannot with finality be designated as any one of the known species. Since this is the situation, it appears better to create the distinctive specific name "*Corinebacterium quasisterium* (Cooper)".

SUMMARY

Morphologic, cultural, chemical and pathogenic studies were made of a gram negative bacillus isolated from the sterile water system of cystoscopic and treatment rooms.

Results of the above studies are recorded and a name applied for the organism, the 'characters' of which do not appear in conformity with any available recorded determinative studies of the Bergey *Manual* or in the literature.

Stock cultures of the organism are preserved on conservation medium and will be supplied to laboratories upon request.

Acknowledgement—It is a pleasure to express gratefulness for the helpful comments of Dr. J. Howard Brown of Johns Hopkins Medical School during the progress of this work. Thanks are also extended to Dr. S. E. Branham and S. A. Carlin of the United States Public Health Service for checking the cultural reactions obtained by the author and to Dr. Hugh H. Young for the cooperation and encouragement of his laboratory and clinical staff. Director McCoy of the United States Public Health Service Laboratories and Captain C. S. Butler, Commanding Officer at the United States Naval Medical School, have kindly extended the facilities of their laboratories in the work of rechecking most of the original findings of the author. Dr. William W. Ford has reviewed the cultures and concurred in the diagnosis.

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THE HISTOLOGIC CLASSIFICATION OF CARCINOMA OF THE CERVIX AS REGARDS ULTIMATE PROGNOSIS*

BY W L McNAMARA, M D, OAK PARK ILL

FROM the time that Virchow gave his conception of a malignant tumor as a growth made up of cells that differ from the normal cells of the body in their rapidity and manner of growth, attempts have been made to classify them based on their histologic characteristics. This was found to be unsatisfactory and no prognosis could be offered from the histologic structure of the tumor, for example, it was found that the prognosis of the breast tumor did not depend upon whether it was classified as an adenomatous, serous or colloidal tumor.

In 1921 Broders and MacCarty offered a classification based upon cell differentiation and reproductive ability of tumor cells. These criteria proved to give a far more satisfactory classification and better possibility of prognosis. On these principles, cancers of the fundus of the uterus have been classified by Mahle, those of the tongue by Simmons, those of the cervix by Martzloff, and those of the lip, skin and genitourinary organs by Broders.

Broders based his classification of epitheliomas of the genitourinary organs on a study of 473 cases observed in the Mayo Clinic from Nov 1, 1904, to July 22, 1915. In 269 of these cases the lesion was in the cervix. He divides these neoplasms into four grades as follows. If the tumor contains about three-fourths differentiated and one-fourth undifferentiated epithelium it is graded 1, if the differentiated and undifferentiated elements are about equal, it is graded 2, while if it shows one-fourth differentiated and three-fourths undifferentiated epithelium it is graded 3, and if there appears to be no attempt at differentiation it is graded 4. When correlated with the clinical history, this grouping proves to be significant, for he finds that the total good results for all organs were 83.33 per cent in Grade 1, 45.90 per cent in Grade 2, 25 per cent in Grade 3, and 12.19 per cent in Grade 4.

We have applied Martzloff's criteria in classification of cancer of the cervix in a number of cases, and found that his classification in too many instances did not harmonize with the end-results. Therefore, we made a study of our cases to determine, if possible, better microscopic criteria as the basis for classification. From this study we have been led to alter some of Martzloff's criteria, and have arrived at a method of classification which gives more satisfactory agreement with the clinical history in some three hundred cases.

Specimens from cases of over three years' duration only were used. If the patient was still alive, it was assumed that she was free from the disease.

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The method of classification follows

Two classes were made, "high" and "low" malignancy. In the "low" group we classified all of the tumors that tended toward keratohyaline formation, in which the cells tended to form keratohyaline material and epithelial pearls. The cells in these cases more or less closely resemble in appearance and arrangement the natural stratified and squamous epithelium of the portiovaginalis of the cervix, and are thus well differentiated. The cells are large, very much of the same size and clear. In many cases the tumor cannot be distinguished from ordinary epidermoid carcinoma of any mucous membrane. In this class we see many "pearls." The more "pearls" the less the malignancy. If a sufficiently large piece of cervix is given the laboratory,

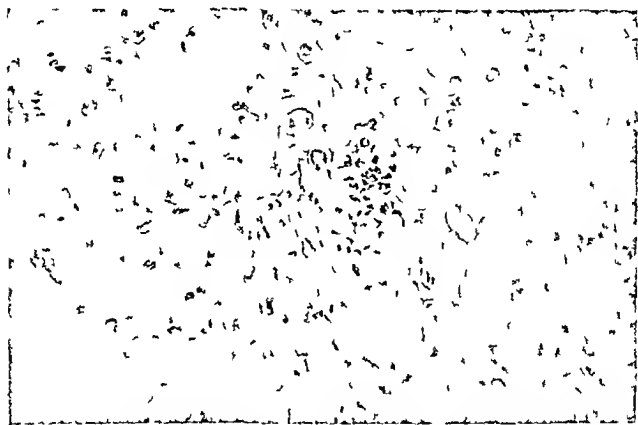


Fig 1—Low type cervical malignancy. There are attempts at keratohyalinization and pearly body formation. The individual cells are larger, clear and resemble the cells of the portiovaginalis.

the tumor sometimes assumes a warty appearance. There is also very little infiltration into cervical tissue.

The group that is classified as of "high" malignancy has the following microscopic appearance. The cells are of two types: the first type of cells are of uniform size, closely packed with a clear cytoplasm and a small round nucleus containing very little chromatin; mitotic figures are few.

The second type of cell in this group resembles a spindle cell. They contain much chromatin, are closely packed, have many mitotic figures and there is never keratohyalinization or the formation of the so-called "pearly bodies." They have the appearance of cells found in the rodent ulcer, although when viewed under high power they are larger, show more mitotic figures, and contain much more nuclear chromatin. They invade profusely

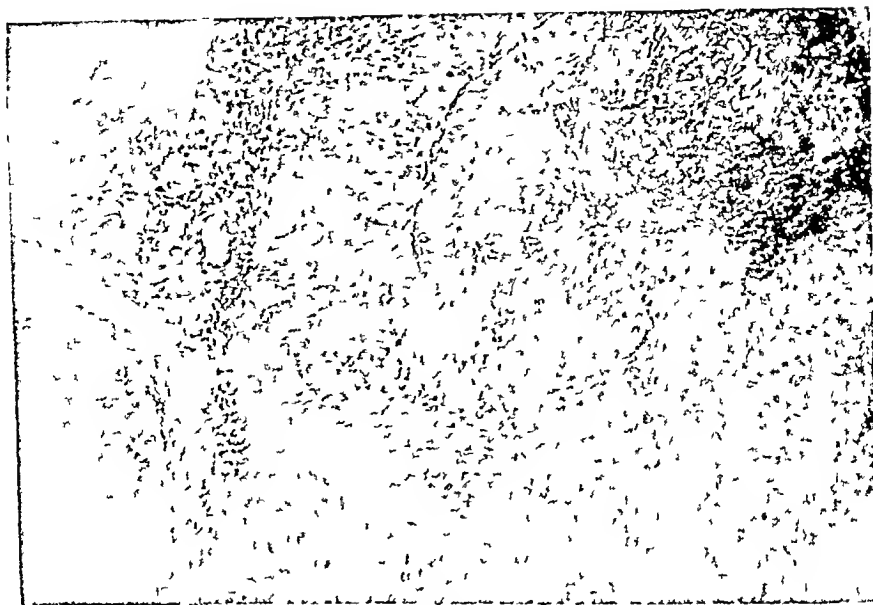


Fig 2— High type cervical malignancy. Spindle-shaped type of cell with dense chromatin, clear cytoplasm, relatively small nucleus and much mitosis.

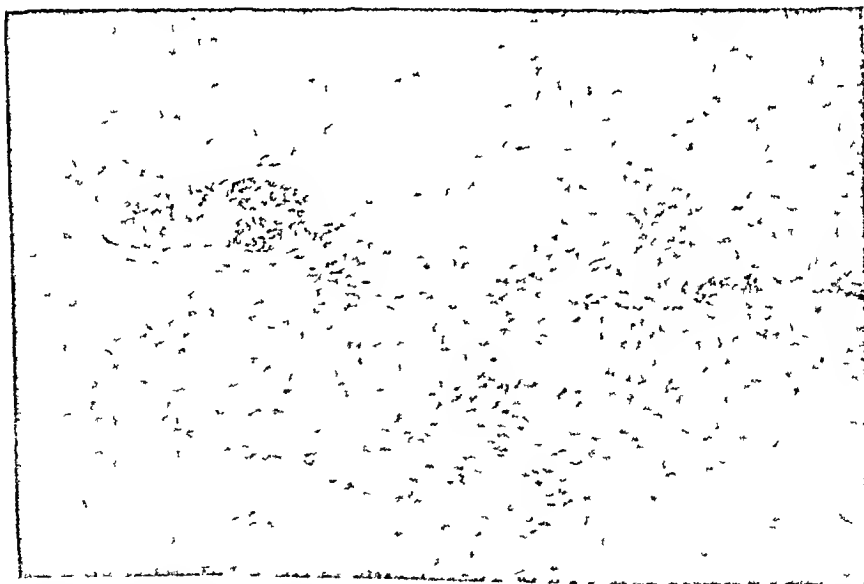


Fig 3— High type cervical malignancy. Round cells usually present with the spindle-shaped cells. These cells are quite large, show relatively little mitosis with little cytoplasm. A smaller amount of chromatin as compared to the spindle shaped cells.

Generally both cell types are found in the same tumor but many times only the latter type are seen. We have never found the first type of cell alone in the tumors studied.

We find another group comprising the adenocarcinoma, which formed a very small portion of our cases and which were relatively nonmalignant in this series. We have accordingly confined our study principally to the squamous cell cancer.

The number of cases studied in each class are as follows. Out of 294 cases we found only 15 of "low" malignancy, 276 of 'high' malignancy, and 3 of adenocarcinomas. Of the "low" malignancies 11 are living except 3 who died from other causes. Therefore we conclude histologically that all cancers of the "low" malignancy group are of a relatively benign character clinically.

The 'high' malignancy class gives an entirely different finding as to end results. After the three year period all cases belonging to this class are dead from cancer. We therefore conclude that in this type of cancer a prognosis can very easily be made from the microscopic slide. It is evident from our findings that a section taken from whole tissue is much better than individual cell description. We have used Wright's new method of acetone fixation and find it wholly satisfactory for immediate diagnosis.

In the adenocarcinoma group we have only three cases and all are living after the three year period. No attempt at classification is made because of the end result obtained.

We have made no attempt to separate the prognosis or end results in treated or untreated cases. The type of treatment, whether it was surgery x-ray or radium gave little difference as regards end results in our cases. We can see no relationship when the end result is already known although the type of treatment may have a great influence in early cases.

CONCLUSIONS

Two hundred ninety four cases of cancer of the cervix were studied microscopically in an effort to determine the degree of malignancy and make some classification suitable to our needs, so that in the future some prognosis might be given. The microscope according to our conclusions, is one of the most valuable assets in the determination of prognosis in malignancy. The squamous cell type of cancer was divided into two types of malignancy which we called "high" and "low".

The 'low' group is made up entirely of large even cells which have a tendency toward 'pearl' formation. The cells as a rule are equal in size and do not infiltrate.

The 'high' group is made up of spindle cells with much chromatin and a small nucleus. They are closely packed giving the appearance of the ordinary basal cell carcinoma. This is the highest type or the most malignant type of cancer found. Along with this squamous cell we usually find a round cell with a clear cytoplasm and a very early chromatinized nucleus. This cell never invades tissue. The cell looks more or less normal. The round cell type of cancer is always mixed with the spindle cell type.

We have no accurate data upon adenocarcinomas. The three cases which we have had in our series of 294 are all living. Our experience with adenocarcinoma does not give any reliable data as to the degree of "high" or "low" malignancy of the disease process.

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THE INFLUENCE OF BLOOD GROUPS IN MALARIAL TRANSFUSIONS*

By E. M. KNIGHTS, PH. D., TOLEDO, OHIO

DURING the year 1928 F. J. Farnell, psychiatrist for the Providence City Hospital, while treating cases of general paresis with injections of human blood containing malarial parasites, noticed that the period of incubation of malaria in the recipients varied and in some instances a single injection of from 2 to 5 cc. of "malarial blood" failed to induce malarial symptoms in the recipient. Furthermore two paretics receiving blood from the same donor at the same time often differed widely in the incubation periods of their malarial symptoms.

Following a discussion of this situation the author asked for permission to type the donors of malarial blood and the recipient paretics, to prepare stained smears of the donors' blood and to prepare smears of the blood of the recipient paretics from time to time.

Early in our study it became evident that the question of blood groups was an important factor in determining the incubation period for malaria after the transfusion of small quantities of malarial blood.

Plasmodium vivax of benign tertian malaria was being used for this work and it is an accepted theory that in man the only time that the parasite is found free from the erythrocytes is during that stage of schizogony when the merozoite ruptures and the merozoites migrate to fresh erythrocytes.

If due to an incompatibility of blood groups between donor and recipient, there is agglutination and lysis of the donor's red cells it is probable that only those malarial parasites which are in the merozoite stage and ready to infest new erythrocytes may survive. If, on the contrary, the donor and recipient are in the same blood group or in such a relationship that the erythrocytes of the donor are not agglutinated by the serum of the recipient we would expect a minimum incubation period.

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Another factor tending to influence the incubation period is undoubtedly the stage of development of the parasite in the blood of the donor at the time the transfusion is made

There was, in nearly every case of successful malarial inoculation, a tendency for double tertian malaria to develop with chills occurring every twenty four hours. In these double tertian cases two distinct stages in the development of the parasite were easily demonstrated in the majority of smears taken.

In cases where an incompatibility of blood groups existed and yet the period of incubation was shorter than might be expected it was often found that there was a predominance of full grown merozoites in the blood of the donor and that the merozoites liberated by destruction of erythrocytes due to incompatibility were ready to attack fresh erythrocytes regardless of blood group.

The following twelve cases are illustrative of the findings in this study.

CASE NO	RECIPIENT'S GROUP MOSS CLASSIFICATION	DONOR'S GROUP	INCUBATION PERIOD (ELAPSED TIME FROM INOCULATION TO FIRST RISE IN TEMPERATURE)
1	IV	IV	4 days
2	II	II	4 days
3	IV	IV	4 days
4	II	IV	10 days
5	IV	II	13 days
6	IV	II	9 days
7	II	III	11 days
8	IV	III	7 days
9	IV	II	4 days
10	III	IV	10 days
11	IV	III	12 days
12	III	II	No evidence after first injection Reinoculated
12	III	II	10 days

In February, 1929, there appeared in the *Archives of Pathology* an abstract of an article by G. Hopf on the "Significance of the Blood Group and of Plasmodium Sporulation on the Type and Incubation Period of Malaria." Hopf's* original article appeared in the October 12, 1928, issue of the *Münchener medizinische Wochenschrift*. In this article Hopf reported a study of 81 cases in which blood groupings were done before transfusions of malarial blood. His findings were the same as ours and certainly more conclusive. However, it is of interest to state that the case records at the Providence City Hospital will show that we started our work in May, 1928, without the knowledge that work was being done elsewhere along the same line.

541 ERIE STREET

LABORATORY METHODS

A REDUCTION IN THE AMOUNT OF BLOOD REQUIRED FOR THE FOLIN MICRO BLOOD-SUGAR METHOD

BY HAROLD J JEGHERS, B S,[†] AND VICTOR C MYERS, PH D, CLEVELAND, OHIO

AT THE time of the publication of Folin's micro blood-sugar method,¹ it occurred to one of us that the amount of blood employed could be reduced from 0.1 to 0.025 c.c. without materially changing the method. At first our hemoglobin pipettes were employed, but later we had pipettes constructed similar to the Folin pipette with the calibration mark at 0.025 c.c. We now use these smaller Folin pipettes for hemoglobin estimation. It is materially easier to obtain 0.025 c.c. of blood from a finger tip puncture, and we believe that our data show that the results are quite as satisfactory.

Observations on twenty three miscellaneous hospital bloods are given in Table I. Determinations were carried out with the modified Folin micro

TABLE I

COMPARISON OF BLOOD SUGAR FINDINGS OBTAINED ON VENOUS BLOOD WITH THE MODIFIED FOLIN MICRO METHOD (EMPLOYING 0.1 AND 0.025 C.C. OF BLOOD) AND THE FOLIN WU AND BENEDICT METHODS

SPECIMEN	MODIFIED FOLIN MICRO METHOD		FOLIN WU METHOD	BENEDICT II METHOD
	0.025 C.C. BLOOD	0.1 C.C. BLOOD		
	mg	mg	mg	mg
1	72	67	67	--
2	72	69	75	69
3	73	75	91	69
4	65	75	80	59
5	72	75	83	71
6	63	76	79	62
7	69	76	78	70
8	74	78	83	70
9	74	78	81	--
10	78	81	85	--
11	76	83	91	69
12	83	84	99	81
13	78	87	91	73
14	84	91	92	85
15	97	94	104	79
16	104	109	112	--
17	111	125	132	109
18	140	142	144	126
19	144	142	146	---
20	221	227	258	---
21	235	239	265	---
22	259	250	263	---
23	367	381	411	---

Last five specimens from diabetics. Analyses made July 2-25 1929

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method² employing 0.025 cc and 0.1 cc of blood and by the Folin Wu and Benedict II method³. With a few exceptions, the agreement between the two micro methods was fairly good, although for some reason the use of 0.025 cc of blood seemed to give slightly lower results, thus more nearly approaching the figures for the true blood sugar as indicated by the Benedict II method. A possible explanation for the slightly lower figures obtained when 0.025 cc of blood was employed is that the greater dilution of the blood with the tungstic acid solution may have led to a greater precipitation of the nonsugar reducing fraction. It is further possible that with substitution of a zinc precipitant for the tungstic acid the method might yield the true glucose content.

Table II gives a few comparative figures on finger blood. The agreement between the two methods is quite good, although with the smaller amount of blood the results are again slightly lower.

TABLE II
COMPARISON OF BLOOD SUGAR DETERMINATIONS ON FINGER BLOOD

BLOOD EMPLOYED	MG SUGAR PER 100 CC						
	NO 1	2	3	4	5	6	7
cc.							
0.025	91	91	95	96	118	132	156
0.1	93	95	97	95	119	132	159

In the Folin micro method the proteins are precipitated with tungstic acid, the sugar in the supernatant solution after centrifuging oxidized with alkaline potassium ferrieyanide, and the ferrieyanide produced measured colorimetrically as Prussian blue. This method is a very delicate one and furnishes an abundance of blue fluid for color comparison, even with 0.025 cc of blood. For this reason it is technically superior to any other micro method.

As originally described, the 0.1 cc of blood after dilution to 10 cc with the tungstic acid solution yields about 9 cc of extract, of which only 4 cc is used and ultimately diluted to 25 cc. Our modification consists in doubling the preliminary dilution, i.e., 0.025 cc to 5 cc employing 4 cc of the extract and a final dilution of 12.5 cc.

As pointed out by Folin,¹ it is essential that the micro pipettes should be perfectly clean to have them draw up the blood by capillary attraction. We always leave them in cleaning fluid overnight.

With the aid of a capillary pipette* calibrated to contain 0.025 cc of blood, allow blood after lancing finger tip to flow to mark or slightly above, adjust level of blood to mark, see that the outside of the pipette is free from blood, and immediately discharge contents into 5 cc of tungstic acid† in 15 cc centrifuge tube. Completely wash blood from pipette by sucking up blood several times, stir with the pipette centrifuge, and pour the supernatant fluid into a clean dry test tube.

*Both the 0.025 and 0.1 cc pipettes have been made for us by Eimer and Amend, N. Y.

†Prepared by diluting 20 cc of 10 per cent sodium tungstate to about 800 cc with distilled water in a volumetric flask, adding 20 cc of 2/3 N sulphuric acid and diluting to volume.

Transfer 4 c.c. of the blood extract to a tube graduated at 125 c.c. To a similar tube add 2 c.c. of standard glucose solution* (0.01 mg glucose per c.c.) and 2 c.c. of water (for diabetic bloods employ 4 c.c. of standard). To both tubes add 1 c.c. of the potassium ferricyanide solution† and 1 c.c. of the cyanide carbonate solution‡. Heat both tubes in a beaker of boiling water for eight minutes. Cool in running water for one to two minutes. Now add 15 c.c. of the acid ferric iron§ to each tube, allowing it to run down the side of the tube to prevent foaming. Mix by gently rotating the tube, let stand for one to two minutes, add 5 c.c. of water, which should bring the volume to the 125 c.c. mark. Mix and make the color comparison.

If trouble is experienced in matching colors due to the yellow color of the ferricyanide (green), this can be compensated for by the use of a yellow light filter. In our work we have used the Klett biocolorimeter and have placed a strip of filter paper saturated with picric acid over the lamp window, following a suggestion made to us by Dr. Malmros before the publication of his paper with Dr. Folin. A suitable glass filter may also be used.

Calculation—Since the standard contains 0.02 mg glucose and the equivalent of 0.02 c.c. ($\frac{1}{50}$ of 0.025 c.c.) of blood is employed, the reading of the standard divided by the reading of the unknown times 100 gives the mg of sugar per 100 c.c. of blood.

CONCLUSIONS

The Folin micro blood-sugar method is modified to employ only 0.025 c.c. of blood. It is much easier to secure 0.025 c.c. than 0.1 c.c. of blood from anemic and emaciated subjects, infants and children. Glucose tolerance tests may be run with less discomfort to the patient. It is a satisfactory routine method for diabetic patients.

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*This contains 0.01 mg. of glucose per c.c. in 0.025 per cent benzoic acid and is prepared from a stronger solution containing 2 mg. per c.c. by diluting 200 times.

†Prepared by dissolving 1 gm. of c.p. potassium ferricyanide in distilled water and diluting to 500 c.c. (If chiefly diabetic bloods are to be examined it probably is preferable to double the strength of the ferricyanide as Folin and Malmros now suggest.) The major part of this solution should be kept in a brown bottle in a dark closet the portion in daily use also being kept in a brown bottle.

‡Prepared by dissolving 8 gm. of anhydrous sodium carbonate in 40 to 50 c.c. of water in a 500 c.c. volumetric flask with the aid of shaking, adding 150 c.c. of freshly prepared 1 per cent sodium cyanide diluting to volume and mixing.

§Ferric iron solution prepared as follows. Suspend in a liter cylinder filled with water 20 gm. soluble gum ghatti on a copper wire screen just below the surface and leave overnight (eighteen hours). Remove the screen and strain through the doubled layer of a clean towel. To this filtrate add a solution of 5 gm. of anhydrous ferric sulphate in 75 c.c. of 85 per cent phosphoric acid plus 100 c.c. of water. Now add a little at a time about 15 c.c. of 1 per cent potassium permanganate solution to destroy certain reducing materials present in the gum ghatti. The slight turbidity of the solution will disappear completely if kept at 37° C. for a few days.

SEROLOGIC STUDIES BY THE PRECIPITATION, PRECIPITATION FIXATION AND THE COLD FIXATION TESTS FOR SYPHILIS*

B1 B S LEVINE PH D, HINES, ILLINOIS

I

THE antigen precipitation reaction in the laboratory diagnosis of syphilis has earned almost universal recognition. Its appeal to the laboratory worker rests on the technical simplicity, resulting from the elimination of the hemolytic system, and in the apparent ease of performance. Its appeal to the physiologic chemist rests on the probability that the flocculation of alcoholic antigen in syphilitic serum is a direct manifestation of the union of antigen with antibody. However, since the antigen employed in the precipitation reaction is derived from the same materials and by practically the same process as the antigen used in the complement fixation procedure, no specificity of reaction bearing upon syphilis from a theoretic consideration can be claimed for antigen precipitation any more than for complement fixation. In either of the procedures the evaluation of the results rests on a purely empirical ground.

I have previously expressed the opinion that the phenomenon of antigen precipitation, like that of complement fixation, is controlled largely by the same conditions that control most colloidal reactions¹. It is greatly influenced by the quantitative relationship existing between the reacting substances present in the serologic system at the time the test is performed. Such relationship varies with each serum tested². It is difficult, therefore, to evolve a sufficient yet simple system of quantitative relationships which would result in complete and proper antigen precipitation in every case.

Parallel studies in complement fixation and antigen precipitation have confirmed this statement. The truth of this assertion becomes particularly evident when the so called combined antigen precipitation and complement fixation tests are performed simultaneously on a large number of sera. Keining³ made such a combined study of the Sachs Georgi test and the Wassermann reaction and found that a positive Sachs Georgi test occasionally yielded a negative superimposed Wassermann test, while on the other hand, a negative Sachs Georgi might be followed by a positive superimposed Wassermann reaction. Kahn, Landau, and McDermott⁴ made a similar study by combining the Kahn procedure with the complement fixation reaction and found a disagreement of 20 per cent in the results.

I undertook a study of the combined complement fixation precipitation reaction comparing it with the cold incubation procedure, a study which had not been made previously. Some interesting and instructive results were obtained. The precipitation tests were carried out according to Kahn⁵. Upon

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the completion of the precipitation tests, the sets were placed in the ice box and the results read the following day. No probable positive cases could be missed by such a procedure. It is believed by some^{6, 7} that in the cold, antigen precipitates in some instances resulting in the introduction of nonspecific positives. I have not found this to be the case, and therefore believe that such a procedure makes reading of the precipitates easier and more certain in the weaker reactions.

Following the period of cold storage complement was added directly to all the tubes of the precipitation tests and the sets incubated at 37° C in the water-bath for one-half hour, the hemolytic system added, and the tubes again incubated for one-half hour. The results were then read and recorded parallel with those of the original precipitation tests. Independent complement-fixation tests were carried out on the same sera using the cold incubation method proposed by Kolmer.⁸ The results were recorded parallel with the findings of the other two tests and a comparison made.

Only cases proved positive by one or more of the procedures studied were considered. Percentages of agreement or disagreement of any group of cases, therefore, bear a relation to the total number of *positive* cases assembled, and to the number of cases of each subgroup, wherever a subgroup analysis was made. Seven hundred cases were studied in this way. Of those, one hundred, or approximately 14.5 per cent were positive by one or more of the procedures used. The 100 cases were subjected to a statistical analysis, summarized below.

COMBINATION I

1 Kahn positive, Kahn Wassermann positive	67.0%
2 Kahn negative, Kahn Wassermann negative	7.0%
3 Kahn negative, Kahn Wassermann positive	23.0%
4 Kahn positive, Kahn Wassermann negative	3.0%

COMBINATION II

1 Kolmer positive, Kahn positive	67.0%
2 Kolmer positive, Kahn negative	30.0%
3 Kolmer negative, Kahn positive	3.0%

COMBINATION III

1 Kolmer positive, Kahn Wassermann positive	91.0%
2 Kolmer positive, Kahn Wassermann negative	6.0%
3 Kolmer positive, Kahn Wassermann doubtful	3.0%

COMBINATION IV

1 Kolmer positive, Kahn positive, Kahn Wassermann positive	64.0%
2 Kolmer positive, Kahn negative, Kahn Wassermann positive	24.0%
3 Kolmer negative, Kahn positive, Kahn Wassermann negative	3.0%
4 Kolmer positive, Kahn negative, Kahn Wassermann negative	6.0%
5 Kolmer positive, Kahn positive, Kahn Wassermann doubtful	3.0%

The following resume presents the analysis more succinctly.

1 Kolmer positive, Kahn Wassermann positive	91.0%
2 Kolmer positive, Kahn positive	67.0%
3 Kahn positive, Kahn Wassermann positive	67.0%
4 Kolmer positive, Kahn positive, Kahn Wassermann positive	64.0%
5 Kolmer positive, Kahn Wassermann negative	6.0%

The above figures indicate that there is a closer agreement between the Kolmer and the Kahn Wassermann than between the Kahn and the Kahn Wassermann, and that the percentages of agreement between the Kahn and the Kolmer, and between the Kahn and the Kahn Wassermann are the same, being 67.0 per cent in either case. Analysis of the subgroup of the Kahn negative and the Kahn Wassermann positive, and of the Kahn negative and the Kolmer positive discloses that in the majority of the cases of these subgroups the intensity of the hemolytic inhibition ranged from "very strongly positive" to "moderately positive." These cases were retested by the antigen and distilled water precipitation procedure described elsewhere, for their ability to fix complement.² They yielded reactions of a high degree of intensity. The conclusion may be drawn from this that in the specimens under consideration the prevailing colloidal conditions were such as to inhibit the separation of the antigen antibody complex. In other words the Kahn precipitation method like other macroscopic methods of precipitation did not prove all sufficient.

On the other hand the Kahn positive and Kahn Wassermann negative, and the Kahn positive and the Kolmer negative cases were few in number and the precipitation in such instances was weak. I hold the opinion at present that the antigen precipitation as finally worked out by Kahn offers a convenient criterion for judging the serologic status of a blood specimen, and that it apparently bids fair to replace the complement fixation procedure in the laboratory diagnosis of syphilis. Nevertheless, the above statistical study indicates that as things now stand the complement fixation procedure involving preliminary incubation in the cold may have some advantages over the macroscopic precipitation method.

It is here frankly admitted that the ultimate decision in any case depends upon the viewpoint of the syphilologist and is based upon his clinical training, observation and diagnostic judgment. It must not be forgotten that only the general mechanisms of complement fixation and of antigen precipitation are plausibly explainable on the basis of the old immunologic conception or of the interplay of physicochemical forces, such as surface tension equilibria, etc., but the particular principles of these reactions which should spell *syphilitic specificity* are entirely unknown. Therefore the correlation of the results of the tests with the viewpoint of one or other group of clinicians concerning the cases under study is at present the *sole* basis upon which the value of any serosyphilitic laboratory procedure is established. Such a basis is strictly empirical and cannot be regarded as scientific. It is accepted or rejected by the clinician in accordance with its agreement or disagreement with his impressions or judgment of any case under consideration.

II

The following briefs of three histories are given as illustrations. The cases are of special interest since they represent the clinical judgment of several specialists men of notable reputation.

CASE 1—

Laboratory Findings—Nov 19, 19— Red blood cell count, 4,160,000 White blood cell count, 5,400 Hemoglobin, 69 per cent Differential, P M N, 41 per cent, S M, 48 per cent, L M, 10 per cent, Trans, 1 per cent

Serologic—Wassermann positive on May 18, 19— Bureau Wassermann 75 per cent Nov 19, 19— Kahn 4 plus Nov 19, 19— Kolmer strongly positive Nov 19, 19—

Physical Examination—No sears noted on penis The scrotal contents seem to be bound together with more or less fibrous nodules of the testicles at their bases The left testicle in the region of the globus minor has a rather large nodule the size of a good cherry It is hard and firm but not tender There is evidence of several old healed sinuses of the left scrotal sac, some of which are due to attempts at aspiration, and others, according to the history, are the results of spontaneous opening and drainage Chest “There are a few medium fine cracklung râles heard above and below the left clavicle on inspiration following expiratory cough No other râles are heard in the chest Whisper and spoken voice sounds show no pathologic changes” (11/20/19—) X ray Report Stereoscope of Chest “Trachea, heart, aorta, and bony framework normal Diaphragm Both costophrenic spaces partly obliterated Lungs The usual calcification and fibrosis at the hila and along all trunks A small cloud and a few thickened trunks in each apex” (11/19/19—) Report of Consultant in Internal Medicine “Percussion shows dullness over both apices, particularly on the left above the clavicle and over the left apex posteriorly râles are heard after coughing No râles were heard over the right The temperature in this case shows a slight rise above normal No specimens of sputum were found positive for acid fast bacilli The x ray plates show distinct involvement of the left apex and slight of the right” (12/10/19—)

Diagnosis—Internist's Tuberculosis, pulmonary, chronic, moderately advanced, active
G U Specialist's Tuberculosis of the epididymis and seminal vesicles

CASE 2—Patient had sore on penis eighteen years ago, treated it himself by the use of rosin pills States the sore healed in about two weeks Denies any skin eruptions, afterward states his glands have never been enlarged except the right inguinal gland which was swollen at the time but declares this was due to injuring it against the corner of the table It was incised by a physician and drained for some little time

Electrocardiographically patient manifests Tachycardia, simple, and somatic tremor

Ophthalmologically patient manifests Retinitis, low grade, secondary, bilateral, choroiditis, circ, disem, bilat, vitreous opacities, bilat, corneal nebula, bilat

Serologic Findings—

Nov 4, 19—	Bureau Wassermann	Negative
	Kahn	Two plus
	Kolmer	Moderately positive
Nov 17, 19—	Bureau Wassermann	25 0 per cent
	Kahn	Three plus
	Kolmer	Moderately positive
Nov 30, 19—	Bureau Wassermann	50 0 per cent
	Kahn	Four plus
	Kolmer	Strongly positive

Proximate Findings—

Dec 5, 19—	Bureau Wassermann	Negative
	Kahn	Negative
	Kolmer	Negative
Dec 9, 19—	Bureau Wassermann	25 0 per cent
	Kahn	Three plus
	Kolmer	Strongly positive

Specialist in Internal Medicine Reports—'The patient's symptoms are entirely out of proportion with what is revealed by the physical examination. I can see no good reason for the extreme dyspnea which he either suffers or affects. There is no evidence of any valvular disease of the heart. The aorta is certainly dilated, as shown in the x ray and as indicated by the character of the second aortic sound. The possibility of myocardial disease must be seriously considered. In view of the history of syphilis it is possible that the patient may have syphilitic disease of the heart muscle and syphilitic aortitis. However this diagnosis is simply presumptive and by no means established. There is nothing to indicate the presence of aneurism.'

Diagnosis Dilatation of aorta Slight enlargement of heart Myocardial disease Emphysema, slight

The Syphilologist Reports—'This patient had a sore on penis eighteen years ago for which he received only local treatment, and subsequent to it had inguinal adenitis. There is no history of treatment for syphilis in his folder. There is a conflict in the laboratory tests. The physical examination is negative for evidence of syphilis. A therapeutic test to determine the possible relation of this infection, if present to the heart disease is recommended. Patient should be given 0.2 gm neosarsphenamine twice a week for three weeks and the effect on the heart noticed.'

Internist's Second Report—'The patient shows nothing in any way different from what was revealed by the examination made previous to the administration of the neosarsphenamine. He has much less dyspnea today than on the previous occasion. There can be no doubt that the patient's symptoms are largely, if not entirely, hysterical in nature. Neither the slight emphysema of the lungs, nor the heart condition could possibly cause the intense dyspnea which the patient exhibits. As far as one can judge from the examination, the treatment with neosarsphenamine has produced no change in the objective findings. I think it is impossible to come to a definite conclusion about the etiology. The evidence of syphilitic infection is certainly inconclusive. The changes in the aorta may be due to a simple arterio-sclerotic process. Diagnoses remain as previously given.'

CASE 3—

Laboratory Findings—

Serologic	Nov 10, 19—	Bureau Wassermann	25.0 per cent
		Kohn	Three plus
		Kolmer	Mod positive
	Nov 19, 19—	Bureau Wassermann	Negative
		Kahn	Two plus
		Kolmer	Weakly positive
P S P	Nov 10, 19—	First hour (intramuscular)	15.0%
		Second hour	25.0%
		Total	40.0%
	Nov 22, 19—	First hour	20.0%
		Second hour	10.0%
		Total	30.0%
Blood Chemistry	Nov 10 19—	N.P.N 48.0	U.A 2.2
	Nov 22 19—	N.P.N 40.8	U.A 2.2
Sugar Tolerance	Nov 26 19—	Before sugar administration	129
		100 grams sugar administered	
		½ hour after	175
		1 and ½ hour after	210
		2 and ½ hours after	220

First Internist Reports—"Examinations here show the kidneys to be more or less seriously involved but to what extent it is impossible at present time to say definitely. There is an increased amount of sugar in the blood, but not enough to seriously handicap the patient at the present time. There is also some nitrogen retention present and the functional test of the kidneys is rather low. The pulse pressure is extremely low." (Nov 26, 19—)

First Psychiatrist Reports—"The patient has clear insight, knows that he has heart disease and that he had difficulty in swallowing and that he had a drooling and paresis of the left face." (Nov 26, 19—)

Diagnosis Facial nerve paresis, second and third branches, left, probably not central, though it may be interstitial.

Recommendation Should be examined more extensively neurologically, after repeated Wassermann, for clearing of diagnosis in this regard.

Second Internist Reports—"The retina shows definite arteriosclerotic changes. The sudden appearance of some paralysis of the left face, tongue, throat, and right hand suggests either a localized edema or the possibility of embolus. Apparently these symptoms have modified to some extent. These first appeared Nov 26, 19—. This would suggest that it was probably localized edema due to the vascular spasm rather than due to embolus." (Nov 30, 19—)

Second Psychiatrist Reports—"The reflexes of the upper extremities are equal and active. The knee and Achilles jerks are active and equal. Plantar response is average. There is no disturbance to light touch or vibratory sense, but the patient has some difficulty in distinguishing sharp from dull over the extremities. The patient was not tested for ataxia or the Romberg position because the least voluntary exertion on his part increased his dyspnea.

"This patient shows no frank paralysis of the muscles that are giving him discomfort. In view of his general circulatory condition, would interpret his trouble as some circulatory dysfunction involving principally the seventh, ninth, tenth, and twelfth nerves." (Nov 30, 19—)

Diagnosis Paresis of left face, tongue, throat, and right hand secondary to his circulatory condition.

Third Internist Reports—"This man presents a picture of congested heart disease. The respiration is Cheyne Stokes variation. There is considerable edema of the feet, legs, flanks, and back. The liver is about four fingers below the costal margin. Lower border of the spleen is palpable. My impression is that there is fluid in the left pleural cavity. Rate is not much accelerated. There is no visible pulsation in the neck. Cardiac impulse is very faint against the hand. Percussion over the heart shows dullness as follows: M R 2d, 5 cm, 3d, 6 cm, 4th, 7 cm, 5th, 8 cm, M L 2d, 6 cm, 3d, 9 cm, 4th, 10½ cm, 5th, 15 cm, 6th, 17½ cm. Shift in right 4th, from left lateral to right lateral posture is 6 cm.

"On auscultation no murmurs are audible. There is not much alteration in the second sound. The sounds of the heart are rather weak, especially in the recumbent posture, though leaning forward cardiac impulse is not strongly felt against the hand, nor is its intensity considerable to the ear." (Dec 6, 19—)

Diagnoses Congestive heart disease associated with arteriosclerosis. Pericarditis with effusion.

Fourth Internist Reports—"The physical examination, as well as the x ray films, indicates a marked enlargement of the heart and the absence of any evidence of obliteration of the cardiohepatic effusion, especially so since there is no pulsus paradoxus and no change in the intensity of the heart sounds with change of position by the patient.

"There is present today a slight edema of the left ankle which has not been present up to now, and it is noteworthy that a few râles can be heard at both bases behind.

"The high blood sugar with fasting stomach, together with the rapid rise in blood sugar following ingestion of 100 gm glucose and the delayed return to normal indicates a definite diabetes. The constant presence of albumin, at times in large amounts, with the presence of casts, together with a high nitrogen blood content and the evidence of retinal hemorrhage and exudate, indicates a definite nephritis.

"The high diastolic pressure when considered with the retinal arteriosclerosis and the evidence of nephritis, could indicate a generalized arteriosclerosis absolutely abnormal for a man of his age" (Dec 13, 19—)

Diagnoses Myocarditis, with hypertrophy, and dilatation of the heart, etiologic factor not found Arteriosclerosis, general Nephritis interstitial, chronic Diabetes, mellitus

Syphologist Reports— "There is nothing in the history to indicate syphilitic infection The physical examination is negative for syphilis except the cranial nerve involvement Definite diagnosis should await spinal puncture" (Dec 14 19—) (Patient refused to submit to spinal puncture)

III

General theoretic considerations and the impressions gained from numerous histories, of which the three cited are offered as examples, lead to the conclusion that to speak of one serologic laboratory procedure as being per se more specific than another is scientific folly However, the cold incubation and the Kahn precipitation procedures belong to the type of immunologic

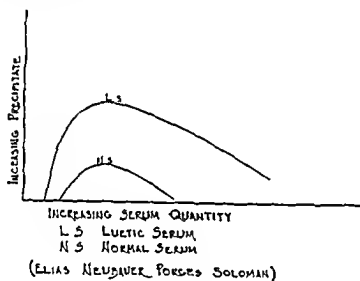


FIG 1

tests which the laboratorian conveniently calls "very sensitive" It was on this basis only that the two procedures were studied comparatively

Porges and Neubauer¹⁰ demonstrated that lecithin and cholesterol are precipitated by a maximum of another colloid An electric neutralization takes place between the oppositely charged colloids and the precipitate occurs when the colloids are in proper balance However, when one of the colloids is in excess of the proper balance, it exerts a protective influence upon the "reaction product" The reversible influence is proportional to the amount in excess A point is finally reached where the precipitate completely disappears They proved further that lecithin and cholesterol are anodic and react in the optimum quantitative relationship with cathodic colloids so as to form a precipitate

These authors in collaboration with Elias and Solomon¹¹ confirmed the fact that the hydrophylic colloid lecithin is electronegative And further, that in its precipitation under the influence of the luetic serum it followed a characteristic curve The curve starts with zero, increases with the volume of the serum added, reaches a maximum and thereafter declines to zero Nor

mal serum also precipitates lecithin, but the zone of precipitation is smaller and the reaction time longer. This is graphically shown in the following diagram

Cholesterol augments the curve of precipitation. Hence the effectiveness of the Kahn antigen in the majority of sera tested. Exceptions, however, occur for the following reasons. First, the electropositive constituents of the many sera are normally variable, second, the degree of positiveness of the sera is of wide range, third, in the Kahn test the antigen is used in three fixed quantities. It is theoretically conceivable, therefore, that in some positive cases the optimum quantitative relationship between the oppositely charged reacting colloids is not obtained by the Kahn test. This may occur in any one or all of the ratios of the Kahn antigen, thus yielding doubtful or false results.

The following table is of interest in this connection

SERUM NO	1	2	3	4	5	6	7	8	9	10
Kahn	214	444	444	444	044	124	440	013	003	003
K Wass	242	204	331	432	122	444	444	432	433	443

It will be observed that in some instances the Kahn was weak or negative while the Kahn-Wassermann was strongly positive, and vice versa. The table also indicates that averaging the readings of the visible precipitates in the three tubes used in the Kahn method in order to deduce an expression of the actual condition in the serum tested appears to be a mistaken procedure.

SUMMARY AND CONCLUSIONS

Seven hundred sera were tested for the presence of antigen combining antibody by the Kahn, Kahn-Wassermann combined, and the Kolmer procedures. One hundred sera, or about 14.5 per cent were positive by one or more of the methods used. The positive values were subjected to statistico-analytic study.

It was pointed out that from a theoretic viewpoint no syphilitic specificity can be claimed for any of the laboratory tests for syphilis now in use.

Excerpts of histories are presented to show instances in which the Kahn and the Kolmer tests gave positive results, yet, the final diagnosis made no reference to syphilis.

The following conclusions are drawn

- 1 The results obtained by the cold incubation and the Kahn-Wassermann fixation procedures closely agree

- 2 The percentage agreement between the Kahn and Kahn-Wassermann, and the Kahn and Kolmer are identical (67.0 per cent)

- 3 The Kahn yields negative results in about 25.0 per cent of the cases where antigen combining substance is present. The Kolmer and Kahn-Wassermann differed in only 5.0 per cent of the cases

- 4 Averaging the three readings of the Kahn precipitates to obtain an interpretation of the results is a mistaken procedure

I gratefully acknowledge the constructive criticism offered by Dr L H Prince, Pathologist at this station, in the preparation of this paper for publication

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A COMPARATIVE STUDY OF 2000 HINTON, KAHN AND WASSERMANN TESTS*

By HLESTER A. AUSTIN, ROCHESTER, N. Y.

IN 1927, Dr. W. A. Hinton¹ of the Wassermann Laboratory of the Massachusetts Department of Public Health described a glycerol-cholesterol precipitation test for syphilis, which has become known as the Hinton test. At this time, he compared the results obtained with his test and the Wassermann test in about 500 consecutive cases, syphilitic and nonsyphilitic. In his opinion, the Hinton test was simple, easily read and sensitive. It apparently possessed greater clinical value than the Wassermann test, employed in his laboratory,² and required less labor, apparatus, and materials. In view of these favorable results, the Hinton test has been employed in our laboratory, during the past year, in parallel with the Wassermann and Kahn tests, which are in routine use here. This paper records the results of that study.

At a symposium on syphilis held at the Boston Dispensary in December, 1927, favorable reports on the sensitiveness and specificity of the Hinton test were given. It was also found to give a larger number of positive reactions in treated cases than the Hinton Wassermann test.^{3, 4, 5}

Smith⁶ and Cheever and Splaine,³ reporting on the Boston Dispensary group of 1610 cases with 2026 serums, found 71.7 per cent agreement between the three tests (1444 serums). Smith⁶ and Munter⁷ reporting on the Peter Bent Brigham Hospital group of 2120 cases with 2331 serums, examined by the Wassermann and Hinton tests, found 90.6 per cent agreement between the tests. In the Massachusetts General Hospital group, Smith⁶ found 80.2 per cent agreement between the Wassermann and Hinton tests in 1759 serums. Smith also reported that in 176 cases from private practice, there was 65.9 per cent agreement between the three tests, there was 74.43 per cent agreement between the Hinton and Kahn tests in this series. In this group there were 17.61 per cent Wassermann positive, 40.34 per cent Hinton positive, and 24.43 per cent Kahn positive reactions, the small percentage of agreement between the three tests was due largely to the great difference in sensitiveness between the Hinton and Wassermann tests. Smith also reported on other hospital groups. The Hinton-Wassermann technique was used by these workers. Smith concluded that as a rule the Hinton test gave a smaller number of so-called doubtful readings, the incidence of false positives was reduced, and it was claimed that the reaction became positive earlier than the Wassermann, and at least as early as the Kahn.

Ferguson and Greenfield,⁸ in examining 460 serums by the three tests found complete agreement in 88.5 per cent, 92.4 per cent agreement between the

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Hinton and Kahn tests, 93 per cent agreement between the Kahn and Wassermann tests, and 91.3 per cent between the Hinton and Wassermann tests. These results were not correlated with clinical data. They concluded that the Hinton test was economical, simple and highly sensitive for syphilitic serums, and that there were indications that the test was somewhat more sensitive and reliable than the Kahn test. They did not think that it was comparable in reliability with a carefully standardized Wassermann technique (they used Medical Research Committee's No. 4 method) but it appeared to pick out certain types of cases, especially "treated" cases and 'latent' cases, where the Wassermann test at times failed. The Hinton test must be reserved as a supplementary test to the Wassermann.

Osmond⁹ found 91.6 per cent agreement between the Wassermann and Hinton tests in 500 serum tests. On 201 other serums examined by the Kahn and Hinton tests, 93 per cent agreement was found, of the 14 disagreeing reactions, 7 were in favor of the Kahn test, and 7 in favor of the Hinton. He concluded that the Hinton test could claim to be the equal at least of the Wassermann and Kahn tests. It was easier to perform and to read than the Kahn test.

Jones¹³ examined the serums from 400 women from a prenatal clinic, 384 serums were negative by both tests, and 7 were positive by both tests, giving 97.75 per cent agreement. Of the remaining 9 serums there were 3 which were Hinton positive Wassermann negative, one was from a treated case of syphilis, one was considered positive from the history and physical signs, the third was considered nonsyphilitic. Of the other 6, which were Hinton negative, 5 gave \pm reactions with the cholesterinized antigen only, the sixth gave a 2+ reaction, all were considered nonspecific. Jones concluded that a routine blood Wassermann test was of definite value on prenatal patients in the diagnosis of syphilis, the value was increased if checked by a precipitation test, the best of which he had found to be the Hinton test.

Todd¹ examined 728 serums, finding 96.84 per cent agreement between the Kolmer Wassermann test and the Hinton test. He concluded that the Hinton test made a superior check test for the complement fixation test, or for one of the other precipitation flocculation tests, and that, if the complement fixation test was to be discarded, the Kline antigen in the Kahn tube test checked by the Hinton test, made a promisingly satisfactory serologic examination for syphilis.

TESTS IN ROCHESTER HEALTH BUREAU LABORATORIES

The tests included in this paper were carried out between August, 1928 and September, 1929, and consisted of two series of 1000 serums each. In the first series serum specimens of sufficient quantity, after the routine Wassermann and Kahn tests were completed, were selected. Some of these were positive and some negative. In the second series, serums which gave positive reactions by either the Wassermann or Kahn test or by both as well as serums from treated cases, negative by both tests were selected. A few routine negatives were included each time tests were set up, as controls. The tests were all carried out with antigen obtained from Dr. Hinton. Between

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The Kahn antigen used in this laboratory is purchased from the Michigan Department of Health. A two tube test is used, omitting the tube containing the largest amount of antigen dilution (0.05 c.c.) This is done in order to secure uniformity, as many serums are not sufficient in quantity for a three tube test, otherwise the test follows the usual Kahn technique.¹¹ About 100 tests are done daily and all tests are read by two workers.

The technique used in the Wassermann test is similar to that used in the New York State Laboratory at Albany. The total volume of the test is 0.5 c.c., i.e., one tenth that of the original Wassermann. All reagents are diluted in accordance with preliminary titrations so that 0.1 c.c. may be pipetted in the test. The complement is the pooled serum of at least 6 guinea pigs, each serum having been previously tested for hemolytic activity, natural antisheep amboceptor, and nonspecific fixability with the cholesterolized antigen. The complement is titrated daily with 5 per cent washed sheep cells sensitized with 2 units of amboceptor and 2 units of complement are used in the test. Each specimen of serum is tested with two antigens: an alcoholic extract of beef heart reinforced with 0.4 per cent cholesterol, and an acetone insoluble extract of beef heart prepared according to Bordet's method. Amboceptor and antigens are supplied by the New York State Laboratory. The tests with both antigens are kept for four hours at 3° to 6° C. for the period of fixation. Sensitized sheep cells are then added, and the tests incubated in the water bath at 37° C. for fifteen minutes (until controls with serum alone, and antigen alone, show complete hemolysis). Readings are then made using a color standard, all tubes showing 2+ or more being centrifugalized, and the percentage of inhibition of hemolysis read from the supernatant fluid. About 150 serums are tested daily. Certain serums from cases with histories suggestive of early syphilis, and others which gave a reaction with the Kahn test were set up with the cholesterolized antigen only, and were given one half hour fixation in the 37° C. water bath in addition to the ice box fixation. Of this number, only 8 specimens gave reactions with the water bath fixation at 37° C., and these were confirmed by the Kahn test, or the Hinton test, or by both. These specimens are indicated in Table VII.

PRESENTATION OF RESULTS

The serums included in the tests presented in this paper came from two main sources: (1) the hospitals of the city which include three venereal disease clinics, and a city venereal disease clinic, and (2) physicians in the city. From the hospital and venereal disease clinic patients, fairly sufficient and accurate histories can be obtained. Some of the history blanks, however, accompanying specimens sent in by physicians gave very little information concerning the patient while others may be completely filled out.

From the data available the 2000 serums have been divided into three classes: (1) those from treated cases of syphilis, (2) those from cases which have been diagnosed as syphilis but in which no history of treatment is given and those from cases particularly early ones, in which history or symptoms suggest syphilis, and (3) those called routine, either because no history was given, or because the history did not suggest syphilis. (Table VII)

It is evident that if more information were available some of the serums classified as untreated, suggestive, and routine might have been included in a different group. The serums in the treated case group were accurately known and the findings of the three tests in this group are most valuable. It is in this treated case group that the Hinton and Kahn tests are claimed to be more sensitive than the Wassermann test.

For the tables which are presented, the following key has been used: Wassermann reactions 2+, 3+, 4+, Kahn reactions 2+, 3+, 4+, and Hinton reactions M and +, have been designated marked (M). Wassermann reactions \pm and +, Kahn reactions \pm and +, and Hinton \pm have been designated partial (P). Negative results have been designated negative (N). The Wassermann, Kahn, and Hinton tests have been called W, K, and H respectively.

Tables I and II show the results of the three tests on the first 1000 serums examined, where there was a large percentage of serums negative by all three tests.

TABLE I

RESULTS OF FIRST 1000 WASSERMANN, KAHN, AND HINTON TESTS. SERUMS SELECTED ON BASIS OF QUANTITY. LARGE PREPONDERANCE OF NEGATIVE REACTIONS.

	WASSERMANN			KAHN			HINTON		
	M	P	N	M	P	N	M	P	N
	125			125			120	0	5
	6				6		2	0	4
	17					17	10	0	7
		1		1			1	0	0
		10			10		7	0	3
		19				19	3	0	16
			3	3			3	0	0
			1		1		0	1	0
			818			818	5	0	813
Totals	148	30	822	129	17	854	151	1	848

TABLE II

PERCENTAGES OF AGREEMENT BETWEEN RESULTS OF FIRST 1000 WASSERMANN, KAHN, AND HINTON TESTS.

Agreements	W AND K AND H			W AND K		W AND H		K AND H	
Marked reactions (M)	122	121	130	131	126	132	143	124	133
Partial reactions (P)	8	9	0	11	16	11	0	10	1
Negative	813	813	813	818	818	813	813	836	836
Total Agreements	943	(94.3%)		960	(96.0%)		956	(95.6%)	
Disagreements									
W M 17 and P 19,									
K neg				36					
K M 3 and P 1,									
W neg				4					
W M 16 and P 19,									
H neg						35			
H M 8 and P 1,									
W neg						9			
K M 5 and P 7,									
H neg								12	
H M 18 and P 0,									
K neg								18	
Total Disagreements	57	(5.7%)		40	(4.0%)		44	(4.4%)	
Total Tests	1000	(100.0%)		1000	(100.0%)		1000	(100.0%)	

Tables III and IV show the results on the second 1000 serums, where a large number of positive serums and serums from treated cases of syphilis were included

The comparison of the results set forth in these tables demonstrates clearly the effect of selection of specimens upon the statistics of agreement between tests. With tests which give very few false positive results, a series

TABLE III

RESULTS OF SECOND 1000 WASSERMANN KAHN, AND HINTON TESTS. SERUMS SELECTED ON BASIS OF HISTORY OF SYPHILIS AND POSITIVE WASSERMAN OR KAHN REACTIONS

	WASSERMANN			KAHN			HINTON		
	M	P	N	M	P	N	M	P	N
	433			433			283	111	39
	25				25		7	11	7
	64					64	1	26	37
		24		24			3	10	11
		9			9		0	3	6
		42				42	0	10	32
			4	4			0	1	3
			2		2		0	0	2
			397			397	3	18	376
Totals	522	75	403	461	36	503	297	190	513

TABLE IV

PERCENTAGES OF AGREEMENT BETWEEN RESULTS OF SECOND 1000 WASSERMANN KAHN, AND HINTON TESTS

Agreements	W AND K AND H			W AND K		W AND H		K AND H				
Marked reactions (M)	412	407	293	458	457	439	285	408	303			
Partial reactions (P)	16	21	135	33	34	26	180	21	126			
Negative	376	376	376	397	397	381	381	445	445			
Total Agreements	804	(80.4%)		888	(88.8%)		846	(84.6%)				
Disagreements												
W M 64 and P 42				106								
K neg												
K M 4 and P 2				6								
W neg												
W M 83 and P 49				132								
H neg												
H M 3 and P 19				22								
W neg												
K M 53 and P 15				68								
H neg												
H M 3 and P 55				58								
K neg												
Total Disagreements				196	(19.6%)		112	(11.2%)		154	(15.4%)	
Total Tests				1000	(100.0%)		1000	(100.0%)		1000	(100.0%)	

comprised largely of specimens giving negative reactions will show a high percentage of agreement among the tests. On the other hand a series composed of specimens selected on the basis of a special origin in this case from syphilitics or because of their special reactions with one or more tests, will bring out most clearly the differing capacities of tests to indicate positive results. In comparing the results obtained in the first series of 1000 unselected specimens with those in the second 1000 selected specimens, it is seen that the agreement between the three tests dropped from 94.3 per cent

to 80.4 per cent, the agreement between the Wassermann and Kahn tests from 96.0 per cent to 88.8 per cent, the agreement between the Wassermann and Hinton tests from 95.6 to 84.6 per cent, and the agreement between the Kahn and Hinton tests from 97.0 per cent to 87.4 per cent.

When the two series are combined, the percentages of agreement between the tests fall into an intermediate position, as shown in Tables V and VI. The agreement among the three tests in 2000 cases was 87.35 per cent, between the Wassermann and Kahn tests 92.4 per cent, between the Wassermann and Hinton tests 90.1 per cent and between the Kahn and Hinton tests 92.2 per cent.

TABLE V
RESULTS OF 2000 WASSERMANN, KAHN, AND HINTON TESTS

	WASSERMANN			KAHN			HINTON		
	M	P	N	M	P	N	M	P	N
	558			558			403	111	44
	31				31		9	11	11
	81					81	11	26	44
		25		25			4	10	11
		19			19		7	3	9
		61				61	3	10	48
			7	7			3	1	3
			3		3		0	1	2
			1215			1215	6	18	1189
Totals	670	105	1225	590	53	1357	448	191	1361

TABLE VI
PERCENTAGES OF AGREEMENT BETWEEN RESULTS OF 2000 WASSERMANN, HINTON, AND KAHN TESTS

Agreements	W AND K AND H			W AND K		W AND H		K AND H				
Marked reactions (M)	534	528	423	589	583	571	428	532	436			
Partial reactions (P)	24	30	135	44	50	37	180	31	127			
Negative	1189	1189	1189	1215	1215	1194	1194	1281	1281			
Total Agreements	1747	(87.35%)		1848	(92.4%)		1802	(90.1%)				
Disagreements												
W M 81 and P 61				142								
K neg												
K M 7 and P 3				10		167						
W neg												
W M 99 and P 68						31		80				
H neg												
H M 11 and P 20												
W neg												
K M 58 and P 22												
H neg												
H M 21 and P 55												
K neg												
Total Disagreements				253	(12.65%)		152	(7.6%)		198	(9.9%)	
Total Tests				2000	(100.00%)		2000	(100.0%)		2000	(100.0%)	

Table VI shows that the agreement between the Wassermann and Kahn tests, and between the Kahn and Hinton tests, is practically the same, while that between the Wassermann and Hinton tests is slightly lower. There are 156 disagreements between the Kahn and Hinton tests, 80 being marked or partial Kahn tests, and 76 marked or partial Hinton tests. Of the 152 disagreements between the Wassermann and Kahn tests, 142 gave marked or

partial reactions by the Wassermann test, and of that 142, 110 were from treated cases of syphilis. The Kahn test gave 7 marked and 3 partial reactions, whereas the Wassermann test was negative. Five of these serums were from treated cases of syphilis. Of the 198 disagreements between the Wassermann and Hinton tests, 167 gave marked or partial reactions by the Wassermann test, of these 167, 122, were from treated cases. Of the 31 serums, giving marked or partial reactions with the Hinton test, and no reaction with the Wassermann test, 25 were from treated cases of syphilis.

TABLE VII
CORRELATION OF RESULTS WITH HISTORIES IN 2000 CASES

660 Treated Cases, 33 0%						
	W		H		K	
Marked	414	62 7%	250	39 2%	342	51 8%
Partial	71	10 8%	129	19 6%	40	6 1%
Negative	175	26 5%	272	41 2%	278	42 1%
	660	100 0%	660	100 0%	660	100 0%

194 Untreated Cases and Cases With Suggestive Histories, 9 7%						
	W		H		K	
Marked	105	54 1%	79	40 7%	07	50 0%
Partial	9	4 6%	24	12 4%	4	2 1%
Negative	80	41 3%	91	46 0%	03	47 9%
	194	100 0%	194	100 0%	194	100 0%

1146 Routine Cases, 57 3%						
	W		H		K	
Marked	151	13 2%	110	9 6%	151	13 2%
Partial	25	2 2%	38	3 3%	9	0 8%
Negative	970	84 6%	908	87 1%	986	86 0%
	1146	100 0%	1146	100 0%	1146	100 0%

One specimen } These specimens gave reactions with the Wassermann test with fixation at
One specimen } 37 C for thirty minutes but not with four hours at 3 to 6 C
Six specimens }

Table VII shows the correlation of results with histories. It shows that approximately the same number of reactions in treated cases of syphilis were obtained by the Kahn and Hinton tests. The Hinton test gave a much larger number of partial reactions. This may be partly accounted for by the fact that the final result of the readings of the two tube Kahn test is higher than with the three tube Kahn test, because the least sensitive tube is omitted. Both tests were far behind the Wassermann test in the number of marked or partial reactions obtained.

These results on treated cases of syphilis were not unexpected, though not in agreement with most published reports of the Kahn and Hinton tests. The difference lies, probably, in the Wassermann technic used. The New York State Laboratory method, which is employed in this laboratory, is a sensitive one, far more so than that used by Dr. Hinton and his associates. This is clearly shown in the report of Gilbert and Langworthy,¹² in which in 1926 seven laboratories conducted tests on 252 serums, each laboratory using

its own Wassermann technic. Among the laboratories were those of Dr Hinton, and the New York State Laboratory at Albany. The Albany laboratory gave 147 marked and partial reactions (with no reactions in control cases) while the Boston laboratory gave only 41. In that same series of comparative tests, Dr Kahn performed his precipitation test on the 252 serums, obtaining only 110 marked and partial reactions, as against Albany's 147 marked and partial reactions with the Wassermann test.

The results shown in Table VII in treated cases, confirm this work. Austin and Frier¹⁴ found that in 393 serums from treated cases of syphilis, the Wassermann test (New York State Laboratory method) was somewhat more sensitive than the Kahn test, giving 89.1 per cent of marked or partial reactions, while the Kahn test gave 86.1 per cent.

TABLE VIII
ANALYSIS OF DISAGREEMENTS IN 253 CASES

DISAGREEMENTS	WASSERMANN			HINTON			KAHN			TYPE OF CASE
	M	P	N	M	P	N	M	P	N	
W (M or P) KH (N)	39	37	0	0	0	76	0	0	76	Treated Cases of Syphilis
W (N) KH (M or P)	0	0	2	0	2	0	1	1	0	
K (M or P) WH (N)	0	0	5	0	0	5	3	2	0	
K (N) WH (M or P)	24	10	0	6	28	0	0	0	34	
H (M or P) WK (N)	0	0	23	6	17	0	0	0	23	
H (N) WK (M or P)	36	10	0	0	0	46	30	16	0	
Total	99	57	30	12	47	127	34	19	133	186
W (M or P) KH (N)	1	2	0	0	0	3	0	0	3	Cases With Suggestive Histories
W (N) KH (M or P)	0	0	0	0	0	0	0	0	0	
K (M or P) WH (N)	0	0	0	0	0	0	0	0	0	
K (N) WH (M or P)	9	1	0	3	7	0	0	0	10	
H (M or P) WK (N)	0	0	1	1	0	0	0	0	1	
H (N) WK (M or P)	6	3	0	0	0	9	8	1	0	
Total	16	6	1	4	7	12	8	1	14	23
W (M or P) KH (N)	4	9	0	0	0	13	0	0	13	Routine Cases
W (N) KH (M or P)	0	0	3	3	0	0	3	0	0	
K (M or P) WH (N)	0	0	0	0	0	0	0	0	0	
K (N) WH (M or P)	4	2	0	5	1	0	0	0	6	
H (M or P) WK (N)	0	0	2	1	1	0	0	0	2	
H (N) WK (M or P)	13	7	0	0	0	20	17	3	0	
Total	21	18	5	9	2	33	20	3	21	44
Grand Total	136	81	36	25	56	172	62	23	168	253

Table VI shows that there was disagreement between the three tests in 253 of the 2000 serums. The records of the large number of treated cases included in Table VIII give it an especial interest as more numerous disagreements between the results of the various tests are to be expected in this group.

If the Wassermann test only had been used, 30 possible marked and partial reactions in treated cases of syphilis would have been missed, if the Hinton test only had been used 127 possible marked and partial reactions would have been missed, if the Kahn test only had been used, 133 possible marked and partial reactions would have been missed. The Hinton test is slightly

more sensitive than the Kahn test, while both are definitely inferior to the Wassermann test in the number of reactions obtained

CASES GIVING POSITIVE REACTION WITH ONLY ONE OF THE TESTS

It seemed worth while to analyze here the routine, and suggestive cases, whose serums gave a marked or partial reaction with only one of the three tests

There were no positive Kahn reactions with serums from cases in these two groups

The Hinton test gave reactions in three such cases as follows

CASE 1—No 20713, J M, male, aged forty nine, single, laborer Serum tested Sept 28, 1928 Wassermann and Kahn tests were negative, Hinton test positive Spinal fluid No 20794 gave a negative Wassermann reaction, and a colloidal gold reading 5555553332 Patient died Sept 30 1928 The autopsy revealed malignant bacterial endocarditis purulent meningitis, old pulmonary tuberculosis with a bean sized cavity in right lung, fractured skull, and subdural clot Blood cultures revealed hemolytic streptococci This case is of interest because Dr Hinton told me that bacterial endocarditis is a disease in which a false positive Hinton reaction may be obtained This reaction may be regarded as nonspecific

CASE 2—No 16491, J G, aged sixty, laborer, with 4 children Tested July 7, 1929 The Wassermann and Kahn tests were negative, the Hinton test positive The diagnosis given was question of arthritis Two specimens of blood from this patient, examined in March, 1928, gave no reaction with the Kahn and Wassermann tests

CASE 3—No 22373, Mrs C B, aged forty eight, housewife, married, with 2 children Tested Oct 19, 1928 The Wassermann and Kahn tests were negative, the Hinton test positive The diagnosis was given as rash No more specimens were received for examination and a letter sent to the physician concerning the patient was not answered

From the information at hand, it is not possible to say whether or not these two Hinton reactions (Nos 2 and 3) are specific

The Wassermann test gave sixteen reactions, unsupported by the Kahn and Hinton tests These were from patients in the routine and suggestive history groups

CASE 1—No 23420, Mrs C B, aged thirty six with 8 children Serum tested Nov 1 1928, gave a \pm reaction with the Bordet antigen only while the cholesterinized antigen, the Kahn and Hinton tests were negative No more specimens were received, and no reply was received from the letter sent to the physician This specimen was sent in for routine examination

CASE 2—No 22914, Mrs J D, aged forty seven widowed with no children Serum tested Oct 26 1929, gave a + reaction with both Bordet and cholesterinized antigens, while the Kahn and Hinton tests were negative The diagnosis was abdominal tumor No more specimens were received, and there was no reply to a letter sent to the physician

CASE 3—No 21980 Mr C H, aged twenty seven laborer single Serum tested Oct 15, 1929, gave a \pm reaction with the cholesterinized antigen only, while the Kahn and Hinton tests were negative The diagnosis was ulcer of the lip No more specimens were received In answer to the letter, the physician said that he regarded the reaction as nonspecific

CASE 4—No 15293, Mr P L Serum tested June 19, 1929 gave a 2+ reaction with the Bordet antigen only while the cholesterinized antigen Kahn and Hinton tests were negative No more specimens were received and physician did not recall patient on Oct 11 1929

CASE 5—No 19409, Mr J T, aged twenty nine, stone cutter, married, with 1 child Serum tested Aug 6, 1929, gave a \pm reaction with the cholesterinized antigen while the Kahn and Hinton tests were negative Two specimens of blood from this patient were examined at the State Laboratory in Albany, one in 1921, and one in 1922, and each gave a 4+ Wassermann reaction Two specimens of spinal fluid examined here in 1929 gave 4+ reactions, 4 specimens of blood gave 4+ reactions, 3 of these specimens of blood gave positive Kahn reactions also The patient's wife's serum gave a 4+ reaction while the child was negative The patient is now in the Rochester State Hospital for the Insane, where he is considered to be syphilitic

CASE 6—No 23189, Mrs C P, aged thirty eight, married, no children Serum tested Oct 30, 1928, gave a + reaction with both antigens, while the Kahn and Hinton tests were negative She had been admitted to the Rochester General Hospital three months after abortion with apparent left tuboovarian abscess and pelvic abscess She had a high temperature which disappeared after the removal of left tuboovarian abscess and drainage of the pelvic abscess She was discharged Jan 14, 1929, with condition improved

CASE 7—No 23462, Mr C W, aged seventy, married Serum tested Nov 2, 1928, gave a + reaction with the cholesterinized antigen only, while the Kahn and Hinton tests were negative The patient had enlarged prostate, arteriosclerosis and phlebitis with swelling and edema of the feet The physician wrote that the symptoms were not suggestive of syphilis, the patient did not return to his care, and a second specimen was not sent in

CASE 8—No 16627, Mr J B, aged forty, chauffeur, single Serum tested July 3, 1929, gave a \pm reaction with the cholesterinized antigen, while the Kahn and Hinton tests were negative There were two recent previous specimens No 15229 (June 17, 1929) gave \pm with the Bordet antigen, and 3+ with the cholesterinized antigen, No 16131 (June 27, 1929) was negative with both antigens Both these specimens had negative Kahn tests The patient was referred from the Rochester General Hospital to the Veterans' Bureau, and is not in the city now

CASE 9—No 16022, Mr R I, aged thirty four, colored, single Serum tested June 27, 1929, gave a + reaction with the cholesterinized antigen only, while the Kahn and Hinton tests were negative A specimen a year previous, No 13484 (June 18, 1928), gave no reaction with the Wassermann and Kahn tests A specimen examined at the State Laboratory in Albany, No 29971, gave no reaction with the Wassermann test The patient is now in the Iola Tuberculosis Sanatorium

CASE 10—No 23194, Miss E H, aged thirty two, single Serum tested Oct 30, 1928, gave a + reaction with the cholesterinized antigen only, while the Kahn and Hinton tests were negative The diagnosis was carcinoma of the cervix A second specimen, No 23777 (Nov 7, 1928), gave no reaction with the Kahn and Wassermann tests

CASE 11—No 22273, Mr A V, aged forty seven, fireman, married with one child Serum tested Oct 18, 1928, gave a \pm reaction with the Bordet antigen, and a + reaction with the cholesterinized antigen, while the Kahn and Hinton tests were negative The diagnosis was psychoneurosis, hypochondria, and question of duodenal ulcer A second blood specimen No 23748 (Nov 6, 1928) and a spinal fluid No 23634 (Sept 24, 1929) gave no reactions with the Kahn and Wassermann tests, and the colloidal gold was negative

CASE 12—No 23821, Mrs E P, aged fifty, no children, worker in cafeteria Serum tested Nov 8, 1928, gave a 2+ reaction with the cholesterinized antigen, while the Kahn and Hinton tests were negative The diagnosis was persistent ulcer of the leg A second specimen No 24373 (Nov 15, 1928) gave a \pm reaction both with the cholesterinized antigen and with the Kahn test The patient had been referred to the physician in connection with compensation, the condition was not regarded as syphilitic

CASE 13—No 11913, Mrs E F, aged forty five, housewife, one child Serum tested May 7, 1929, gave a 3+ reaction with cholesterinized antigen only, while the Kahn and

Hinton tests were negative. The diagnosis was brachio in a woman of forty five with one healthy child three or four years of age. The previous specimens No 9345 (April 10, 1929) gave a 3+ reaction with the cholesterinized antigen and a negative Kahn test, and No 10459 (April 22 1929) gave a 4+ reaction with the cholesterinized antigen and a negative Kahn test. A specimen tested at the State Laboratory No 19351 (May 23 1929) gave a \pm reaction with the cholesterinized antigen only. The physician said there was no clinical evidence of syphilis, and did not treat the patient for syphilis. Another physician might have concluded differently, the case may be regarded as questionable.

CASE 14—No 22145, Mr A K, aged fifty four, laborer, single. Serum tested Oct 18 1928, gave a \pm reaction with the Bordet antigen and 3+ with the cholesterinized antigen while the Kahn and Hinton tests were negative. The diagnosis was arthritis. Two previous specimens from this patient gave reactions with the Kahn test also. No 20923 (Oct 3 1928) gave \pm with the Bordet antigen 3+ with the cholesterinized antigen \pm with the Kahn test. No 21345 (Oct 8 1928) gave no reaction with the Bordet antigen \pm with the cholesterinized antigen + with the Kahn test. A succeeding specimen No 22350 (Sept 12, 1929) gave a 2+ reaction with the cholesterinized antigen only and a negative Kahn test. Patient received treatment at a venereal disease clinic.

CASE 15—No 12585, Mrs E D, aged twenty two, housewife. Serum tested May 14, 1929 gave 3+ reaction with Bordet antigen and 4+ with the cholesterinized antigen, while the Kahn and Hinton tests were negative. Two previous specimens were received. No 10731 (April 24 1929) which gave a 3+ reaction with the Bordet antigen and 4+ with the cholesterinized antigen and No 11416 (May 2 1929) which gave 2+ with the Bordet antigen and 4+ with the cholesterinized antigen. The patient was pregnant and has been receiving treatment in a venereal disease clinic since that time. The baby's blood test No 21203 (Aug 28 1929) was 2+ with the Bordet antigen and 4+ with the cholesterinized antigen. The Kahn test was negative on No 10731, No 11416, and No 21203.

CASE 16—No 18967, I N, child, aged eight years. Serum tested Aug 1 1928 gave + reaction with the Bordet antigen only, while the cholesterinized antigen the Kahn and Hinton tests were negative. Two previous specimens were examined. No 11736 (May 7 1929) and No 17886 (July 19, 1929) giving 3+ reactions with the Bordet antigen only. Two succeeding specimens No 22147 (Sept 9 1929) and No 24857 (Oct 7 1929) gave reactions with the Bordet antigen only. These four specimens were negative with the cholesterinized antigen and the Kahn test. The child is now under treatment for syphilis. It has not been possible to obtain a specimen of blood from the parents of this child.

Of these 16 cases, therefore, 7 (Nos 3, 6, 7, 9, 10, 11, 12) may be considered nonspecific. 2 (Nos 8 and 13) as questionable. 4 (Nos 5, 14, 15 and 16) as syphilitic. and from 3 (Nos 1, 2 and 4) no further specimens or information were available. Six of the 7 nonspecific reactions were partial (+ or \pm) while the seventh serum gave a 2+ reaction. This was confirmed by a second specimen which gave a \pm reaction with the cholesterinized antigen.

SUMMARY

Below is a comparison of the results of the tests in this laboratory and those of the other workers mentioned in this paper.

PERCENTAGES OF AGREEMENT BETWEEN THE WASSERMANN, KAHN AND HINTON TESTS

Cheever (Boston Dispensary)	71.7 %	2026 serums
Smith (Private practice)	65.9 %	176
Ferguson and Greenfield	85.5 %	400
Rochester Health Bureau Laboratories	87.3 %	2000

PERCENTAGES OF AGREEMENT BETWEEN WASSERMANN AND HINTON TESTS

Hinton	89 1 %	506 serums
Munter (Peter Bent Brigham)	90 6 %	2331 "
Smith (Massachusetts General)	80 2 %	1759 "
Ferguson and Greenfield	90 0 %	612 "
Osmond	91 6 %	500 "
Jones	97 75 %	400 "
Todd	96 84 %	728 "
Rochester Health Bureau Laboratories	90 1 %	2000 "

PERCENTAGES OF AGREEMENT BETWEEN KAHN AND HINTON TESTS

Smith (Private practice)	74 43 %	176 serums
Ferguson and Greenfield	92 4 %	460 "
Osmond	93 0 %	201 "
Rochester Health Bureau Laboratories	92 2 %	2000 "

PERCENTAGES OF AGREEMENT BETWEEN KAHN AND WASSERMANN TESTS

Ferguson and Greenfield	93 0 %	460 serums
Rochester Health Bureau Laboratories	92 4 %	2000 "

CONCLUSIONS

The Hinton glycerol cholesterol agglutination reaction compares favorably with the Kahn precipitation test in treated cases of syphilis, in untreated cases of syphilis and in cases with suggestive histories. It is as simple to perform as the Kahn test and perhaps easier to read. The Kahn test has the advantage of requiring less time to complete the test.

The New York State Wassermann test is more sensitive in treated cases than either the Hinton or Kahn test. This greater sensitivity far more than offsets its rare, nonspecific partial reactions. The Hinton test is much easier to perform, and requires fewer and less expensive reagents and apparatus than the Wassermann test.

The Hinton test might be substituted for the Kahn test but not for a sensitive, carefully standardized Wassermann technic.

Note on Interpretation of Results of Hinton Tests—The objective reading of the results of the Hinton Tests recorded in this paper is thought to be entirely in accord with the practice in Dr Hinton's laboratory. In reporting and evaluating the results, however, a method of interpretation is employed by Dr Hinton which has not been followed in this study. Dr Hinton, who has seen the manuscript of this paper, has offered the criticism that some differences in the percentages of agreement among tests and in the correlation of results with clinical histories might have been obtained if his system of interpreting the results had been used. The following paragraphs, quoted by permission, from a letter from Dr Hinton dealing with this question are set forth here in justice to his point of view.

"The changes observed in the three tubes containing the individual specimen are recorded in a column as — (negative), W (weak reaction), M (moderate reaction), or S (strong reaction), as the case may be. In a separate column we indicate the significance which these readings have as to the presence or absence of syphilis, and this is our 'interpretation'.

"The reactions observed and recorded as — in the three tubes are interpreted as negative. Reactions observed and recorded as W in one

or more tubes, but no stronger, are interpreted as doubtful. Reactions observed and recorded as M or S in any or all of the tubes are interpreted as positive.

"Our 'interpretation' is based on a careful clinical study of many cases and is the report which is given to the clinician as positive, negative, or doubtful. Thus, the clinician gets no idea of the readings, although we have indicated in our records the intensity of the reaction as W, M, or S. Our reason for reporting in this way is that we imply no quantitative significance by these terms, inasmuch as we have found them insignificant in therapeutics, or in diagnosis except to indicate the presence of syphilis."

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CLINICAL AND SEROLOGIC COMPARISON OF THE MICROSCOPIC SLIDE PRECIPITATION TEST FOR SYPHILIS AND THE WASSERMANN TEST WITH THE SAME ANTIGEN*

By B S KLINE, M D, AND S LITTMAN, M D, CLEVELAND, OHIO

IN A SERIES of 9000 microscopic slide precipitation tests and somewhat fewer Wassermann tests with the same antigen, the precipitation test was found to be more sensitive than the complement-fixation test in all stages of syphilis

The microscopic slide precipitation test for syphilis employed in this study was described by Kline and Young¹ It is easier to perform accurately than the Kahn tube test It is simpler in detail and requires less serum, less apparatus and less time The reactions are somewhat stronger in the slide test and the results, magnified about 100 times by the microscope, are much easier to read than those of the Kahn test

The Wassermann test was done by the Cleveland method² It employs overnight primary incubation at 8° C to 12° C, 5 to 10 units of amboceptor and 2 units of complement In a comparative study of 1000 tests by Lyne³ the Cleveland-Wassermann test and the Kolmer-Wassermann test were found to be of equal sensitivity and specificity

The antigen employed for both precipitation and complement-fixation tests was that described by Kline⁴ It is a lipid obtained from chilled absolute alcoholic extract of beef heart powder by precipitation in acetone at 50° C to 37° C

The emulsions for the precipitation test, prepared from Kline antigen, in contrast to Kahn antigen dilutions, are (a) relatively stable, retaining their antigenic properties undiminished for two days, (b) more sensitive than the standard Kahn antigen dilutions,^{4 5} (c) always free of clumps with non-syphilitic sera at low temperatures as at ordinary room temperature, (d) uniform in quality and quantity containing numerous discrete fine particles resembling those of a negative reaction, whereas Kahn antigen dilutions vary in content and contain clumps of undispersed particles resembling those of a positive reaction

The emulsions for the Wassermann test were prepared by proper dilution of the emulsions for the precipitation test

Table I gives the clinical evaluation of the two tests

*From the Laboratory Department and the Department of Syphilology of the Out-patient Clinic Mount Sinai Hospital

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TABLE I

CLINICAL EVALUATION OF MICROSCOPIC SLIDE PRECIPITATION TESTS AND WASSERMANN TESTS WITH THE SAME ANTIGEN

DISAGREEMENTS													
POSITIVE REACTIONS—NO EVIDENCE OF SYPHILIS													
TOTAL TESTS	TESTS IN CASES WITH HISTORY UNOBTAINABLE OR DATA INSUFFICIENT OR SYPHILIS DOUBTFUL	AGREEMENT	TOTAL DISAGREEMENT	FALSE NEGATIVES						WASSER MANN TEST			
				PRIMARY		TREATED SYPHILIS				SLIDE TEST		WASSER MANN TEST	
				UNTREATED	TREATED	SECONDARY	TERMINAL	CONGENITAL	NOT DETERMINED	SENSITIVE	WASSERMAN SENSITIVE	SENSITIVE	WASSERMAN SENSITIVE
9001 (1801 syphilitic sera ± to +++ in slide Wassermann or both tests 20.8%)		76	8829 98.92%	96 1.05%	0	3 0.03%	21 0.24%	55 0.61%	3 0.03%	1 0.01%	3 0.03%	10 0.11%	++

(a) About 3% of tests with 0.2% cholesterolized antigen about 65% with 0.3% cholesterolized antigen
(b) About 33% of tests with noncholesterolized antigen about 65% with 0.6% cholesterolized antigen
(c) + + + + + test in syphilitic serum = agreement
(d) + + + + + test in syphilitic serum other test + + + + + = false negative
(e) + + + + + test in nonsyphilitic serum = false positive

Table I shows the greater sensitivity of the microscopic slide precipitation test in all stages of syphilis. Positive reactions in cases showing no evidence of syphilis were very few and probably due to technical errors.

Table II shows the comparative sensitivity of the two tests in primary syphilis before treatment.

TABLE II

COMPARISON OF SLIDE PRECIPITATION TEST AND WASSERMANN TEST IN CASES OF PRIMARY SYPHILIS BEFORE TREATMENT

DATE	SLIDE PRECIPITATION TEST		WASSERMANN TEST		CLINICAL DATA
	SENSITIVE	VERY SENSITIVE	SENSITIVE	VERY SENSITIVE	
11/11/27	+	++	-	-	Case 1—(J. A.) Chancre seven days' duration
2/16/29	-	±	-	-	Case 2—(J. C.) Chancre seven days' duration Fontana stain negative for Spirocheta pallida, positive for Spirochete vincenti
2/21/29	+	++	-	-	
4/ 1/29	++++	++++	++++	++++	Typical chancre, enlarged inguinal nodes on right side, maculopapular syphilides
4/ 6/29	++++	++++	++++	++++	

Table II shows the greater sensitivity of the precipitation test in primary syphilis before treatment.

TABLE III

COMPARISON OF SLIDE PRECIPITATION TEST AND WASSERMANN TEST IN A CASE OF TREATED SECONDARY SYPHILIS

DATE	SLIDE PRECIPITATION TEST		WASSERMANN TEST		CLINICAL DATA
	SENSITIVE	VERY SENSITIVE	SENSITIVE	VERY SENSITIVE	
5/21/27					Case W. D. Chancre two months' duration Wassermann test reported positive in May at City Hospital. Patient received three injections at City Hospital.
6/ 2/27		++++	++++		
7/11/27		++++	+++		
9/23/27	+	++++	-	+++	
10/20/27	+	+++ +++++	-	-	
11/17/27	+	++	-	-	
2/18/28	+	+	-	-	
8/23/28	++++	++++	++	++++	Clinical recrudescence
9/13/28	++++	++++	++	++++	
10/25/28	+++	++++	-	+++	
11/20/28	+	++	-	++	
4/11/29	±	++	-	-	
4/18/29	±	++	-	-	
5/ 2/29	-	±, +	-	-	
8/10/29	±	+	-	-	

Table III is a representative protocol showing the comparative sensitivity of the two tests in secondary syphilis

Table III shows the greater sensitivity of the slide precipitation test in secondary syphilis

Table IV illustrates the comparative sensitivity of the two tests before and following treatment early in syphilis

TABLE IV

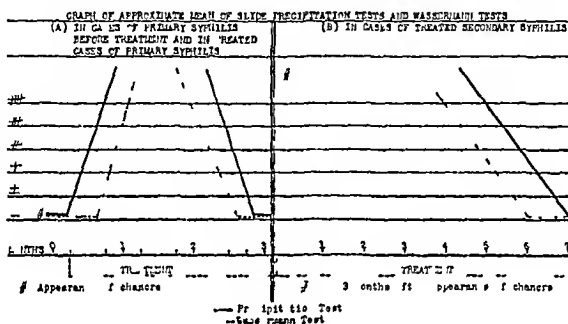


Table IV shows the greater sensitivity of the precipitation test in primary syphilis before treatment and following it, and in secondary syphilis following treatment

Table V is a representative protocol showing a comparison of the two tests in treated syphilis

TABLE V

PROTOCOL SHOWING GREATER SENSITIVITY OF SLIDE PRECIPITATION TEST IN CASES OF TREATED SYPHILIS (5/11/28)

PATIENT	STAGE OF SYPHILIS	SLIDE PRECIPITATION TEST		WASSERMANN TEST	
		SENSITIVE ANTIGEN	VERY SENSITIVE ANTIGEN	SENSITIVE ANTIGEN	VERY SENSITIVE ANTIGEN
D B	3	++++	++++	+++	++++
G W	3	++	+++	-	++
B B	2	-	-	-	-
M L	Cong	+++	++++	++	++++
A. M	3	++++	++++	-	++++
T S	3	++	+++	-	+++
C B	3	++++	++++	+++	++++
A G	3	+++	+++	-	-
G P	3	++++	++++	++	++++
M C	3	+++	++++	++	++++
J N	3	++	++++	-	++
L C	3	++	+++	+++	++++
L A	2	+	+++	-	+++
Total positive reactions		11	12	6	11
Total doubtful reactions		1	0	0	0
Total negative reactions		1	1	7	2
Total tests		13	13	13	13

The following table shows the serologic comparison of the two tests

TABLE VI

COMPARISON OF S708 MICROSCOPIC SLIDE PRECIPITATION TESTS AND WASSERMANN TESTS WITH THE SAME ANTIGEN

	SLIDE TESTS AND WASSERMANN TESTS WITH VERY SENSITIVE ANTIGEN EMULSION					SLIDE TESTS AND WASSERMANN TESTS WITH SENSITIVE ANTIGEN EMULSION				
	AGREEMENT	RELATIVE AGREEMENT	DISAGREEMENT		TOTAL TESTS	AGREEMENT	RELATIVE AGREEMENT	DISAGREEMENT		TOTAL TESTS
			POSITIVE SLIDE, NEGATIVE WASSERMANN	POSITIVE WASSERMANN, NEGATIVE SLIDE				POSITIVE SLIDE, NEGATIVE WASSERMANN	POSITIVE WASSERMANN, NEGATIVE SLIDE	
Tests	7968	509	166	65	8708	7530	633	317	43	8523
Per cent	91.50	5.84	1.91	0.75		88.35	7.43	3.72	0.50	
Tests	8477		231		8708	8163		360		8523
Per cent	97.34		2.66			95.78		4.22		

Evaluation of results —

Positive Reaction = ++++ + + + and + +

Doubtful Reaction = + and ±

Agreement = positive negative or doubtful by both methods

Relative agreement = positive or negative by one method and doubtful by the other

Disagreement = positive by one method and negative by the other, and vice versa

Table VI shows the greater number of positive precipitation tests and the closer agreement of the very sensitive tests with each other than the sensitive tests with each other

DISCUSSION

The Wassermann test requires the careful preparation of five main ingredients: antigen, patient's serum, complement (from guinea pig), red blood cell suspension (from sheep), and amboceptor (from rabbit immunized against sheep red blood cells). The precipitation test requires but two main ingredients, antigen and patient's serum.

The precipitation test is much simpler in detail than the Wassermann test and offers less opportunities for error and less technical difficulties. The precipitation test in other words is easier to perform accurately than the Wassermann test. Furthermore, the precipitation test is more sensitive than the Wassermann test with the same antigen. This is apparently due to the fact that concentrated ingredients are used whereas in the Wassermann test the antigen is greatly diluted and the serum somewhat diluted before the reaction occurs.

The sensitive slide test, more sensitive than the very sensitive Wassermann test, with but one false positive reaction in 8897 tests, is especially valuable in that a ++ or stronger reaction is practically diagnostic of syphilis. The very sensitive slide test is especially valuable in that when negative it rules out syphilis except in a very small percentage of cases.

CONCLUSION

In a series of 9000 microscopic slide precipitation tests and somewhat fewer Wassermann tests with the same antigen, the precipitation test was found to be more sensitive than the complement fixation test in all stages of syphilis

We wish to acknowledge the excellent technical assistance of Miss M G Bowman, A B, in the performance of the majority of the tests reported in this study

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A ROUTINE BLOOD CHEMISTRY UNIT*

BY E G SCHMIDT, PH D, BALTIMORE, MD

THE advent of clinical chemistry as an important diagnostic factor in medicine and surgery necessitates simplified methods and apparatus for the routine determination of the various constituents of the blood and urine. In the hospital where a number of individuals work interchangeably particularly in regard to emergency examinations, a permanent unit for the routine blood chemistry determinations which is practically error proof is very desirable. During the last few years a unit has been in use in the Mercy Hospital laboratory which has aided materially in promoting efficiency, economy and accuracy of results, with the consequent elimination of errors and of confused blood specimens. Essential details of the set up are illustrated in Fig 1 which is practically self explanatory and which was kindly taken for us by Mr Carl Clark of the Art Department of the University of Maryland School of Medicine.

The essential feature of the apparatus, besides the burette system is several rows of solid glass rods driven firmly into holes which have been bored into the solid board background at a slight angle. Upon these solid glass pegs, which are about 5 inches long and $\frac{3}{8}$ inch in diameter, are placed the

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various tubes used in the blood chemistry determinations Each row of tubes, reading from the top downward, is labeled at the left of the board, e g, non-protein nitrogen, creatinine, uric acid, "urea incubation," "urea nesslerization," blood sugar, etc Below the lip of each tube is placed a strip of adhesive tape upon which is written with a glass-marking pencil the number of the tube Each row of these tubes is numbered from left to right in numerical sequence, the last tube on the right is marked "S" and is used for the standard solution In the solid filter-board a number of holes are bored, and numbered to correspond with its blood specimen and the glass peg above it

The complete blood chemistry is carried out in the following manner A number of small 2 oz wide-mouth bottles containing 1 cc of a 1 per cent solution of lithium-, potassium-, or sodium oxalate are baked in an oven overnight and as a result the oxalate is left in a finely divided condition which

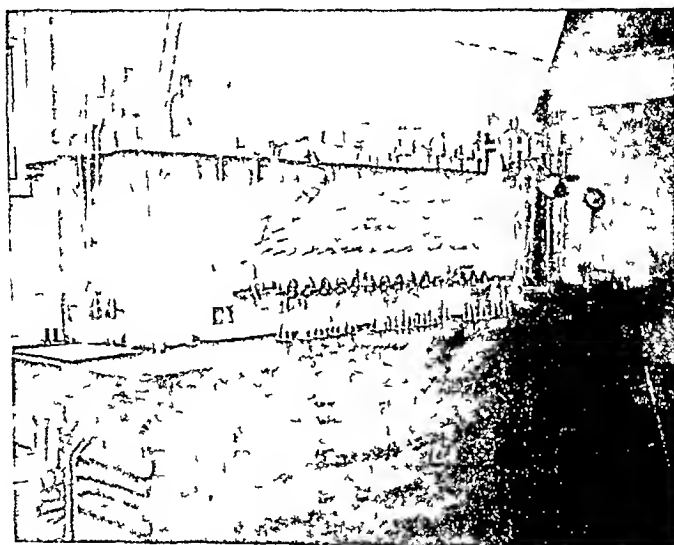


Fig 1

materially enhances its anticoagulative effect About 6 cc of blood is obtained from each patient in the oxalate bottles in the usual manner The specimen bottles labeled with the name and number of the patient are arranged in a row before the apparatus, one bottle on each filter hole Usually 5 cc (1 volume) of blood are accurately pipetted into an Erlenmeyer flask to which 40 cc (8 volumes) of 0.12 N sulphuric acid¹ are immediately added from the burette at the left of the board The contents of the flask are shaken with a rotary motion and some of the solution is drawn into the pipette and then allowed to drain out again Thus each blood is acidified and its empty blood container placed in sequence at the back of the board The pipettes are then blown out and placed on glass wool in a large jar containing a solution of chromic acid cleaning solution² Each Erlenmeyer flask is now given a final inspection and if all the blood is hemolyzed then 5 cc (1 volume) of 10 per cent sodium tungstate is added from the burette just to the

left of the board. The flasks are given a vigorous rotary whirl to aid protein precipitation, but it is not necessary to stopper the flasks when shaking as in the original Folin Wu method.² The contents of the flask are allowed to stand about five minutes, with an occasional shake, and then poured upon the filters which are also in numerical sequence. If the bloods were completely hemolyzed before the addition of the sodium tungstate the filtrates will invariably come through water clear.

The following analytical procedures are used: blood sugar by the method of Benedict,⁴ nonprotein nitrogen and creatinine according to Folin Wu,³ amino acid nitrogen according to Folin⁵ and to Schmidt, uric acid by the method of Benedict,⁶ and the blood urea by the method of Karr.⁷ This direct nesslerization method for the determination of blood urea will be described somewhat in detail, because it is an essential part of the blood chemistry and fits in well with the above described unit.

Into each tube numbered in red and labeled "urea incubation" are placed 5 cc of the corresponding protein free filtrates and into the standard urea tube marked "S" is placed 5 cc of the standard urea solution containing 0.075 mg urea nitrogen. Two drops of a phosphate buffer solution⁸ are added to each tube. Then 1 cc of a concentrated glycerol urease preparation is diluted to 10 cc with distilled water and 1 cc of the enzyme solution is added to each tube.⁹ The diluted urease solution should be prepared daily, but the concentrated glycerol solution retains its full activity for at least a year. The tubes are now incubated for five minutes at 40° to 50° C, and the contents are then quantitatively transferred to the corresponding tubes numbered in blue and labeled "urea nesslerization." This quantitative transfer is carried out in such a manner that the contents of the tubes now have a volume of about 20 cc. Now 2.5 cc of Nessler's reagent⁹ are added to the standard urea tube and the contents diluted with distilled water to 25 cc and mixed by inversion. The solution is now placed in the cups and the colorimeter adjusted for proper readings. Then 2.5 cc of Nessler's reagent are added to tube Number 1 and the contents are diluted to 25 cc, mixed by inversion, and matched at once in the colorimeter. The filtrates are nesslerized and matched singly because a precipitate rapidly forms upon nesslerization which tends to vitiate the results. The tubes are immediately rinsed out with distilled water and replaced upon the glass pegs in numerical order by the analyst who carried out the determination. The "urea incubation" and "urea nesslerization" tubes must never be used interchangeably, and if Nessler's reagent or any mercury solution gets into the incubation tubes, sufficient mercury will be adsorbed to inactivate¹⁰ the urease and as a result the urea will be low and incorrect. When this happens the tubes must be rinsed out with dilute acid and with water several times in order to completely remove the adsorbed mercury.

SUMMARY

A mechanical unit is described for the routine determination of blood chemistries in the hospital laboratory. The use of this apparatus in the deter-

mination of blood urea by the direct nesslerization method is described somewhat in detail

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A NEW MICROCOLORIMETER*

BY A G SHEFTTEL, M D, NEW YORK CITY

THE number of physicians who are doing their own laboratory work, especially blood sugars is increasing rapidly. A colorimeter is required for most of the blood analyses, but the standard colorimeters are quite expensive, and as most physicians need a colorimeter for only one or two items in which they are particularly interested, they use either the dilution method (principle of Sahli hemoglobinometer) or a series of colored tubes filled with colored liquid and used as standards. The disadvantage of the dilution method is that the dilution must be done very slowly, drop by drop, and there is always a certain loss of liquid entailed by the necessary shaking up of each dilution. If a few drops of water are involuntarily added, making the liquid to be examined lighter than the standard, the entire procedure must be repeated. As to the method which employs standards made of a series of tubes filled with colored liquid, the disadvantage is that it is almost impossible to have a liquid standard which does not fade somewhat with time. The most ideal standards are therefore those which are freshly made up and each time from the substance to be examined, or one made out of colored glass which will retain its color permanently.

The colorimeter herein described, like all standard colorimeters, is based on Beers' law which states that light in passing through a colored medium is absorbed in direct proportion to the concentration of colored substance, and it consists of two opaque tubes (Nos 1 and 2) of the same diameter, both having transparent glass bottoms. Underneath these tubes there is a mirror (4) reflecting light. Tube 1 is connected at the lower end with a 1 cc tuberculin hypodermic syringe³ by means of which the liquid to be examined can be withdrawn and then added at will, thus lowering or raising the level of the liquid in Tube 1, and so decreasing or increasing the intensity of color of

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the liquid when examined from the top of the tube. When the color in Tube 1 matches the color of the standard, the percentage of the unknown substance can be ascertained from the divisions on the syringe. Tube 2 is for a standard solution, which can be made fresh each time from the substance to be examined, or the tube can also be made with a colored glass bottom to be used as a permanent standard. Directly under both tubes a yellow colored glass (5) can be inserted to facilitate the matching of colors when the new Folm micro method for sugar in blood is used. This yellow glass acts as a filter in removing the yellow color, which is due to an excess of potassium ferrioxanide in the reagent.

The colorimeter is used in the following manner. For instance we want to test sugar in the blood. After the substance to be tested is transformed into a colored compound and properly diluted 2 c.c. of this liquid is poured into Tube 1 and 2 c.c. of the freshly made standard representing 0.1 per cent

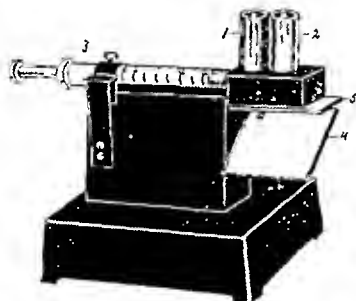


Fig. 1

sugar, is poured into Tube 2, or a permanent glass standard may be used instead. If the solution to be tested is lighter than the standard this indicates that the blood contains less than 100 mg. of sugar per 100 c.c. In this case add another c.c. of the unknown colored compound to Tube 1. By moving out the piston of the hypodermic syringe, the level of the liquid is lowered and the depth of the color diminished until both colors match. All shades can be obtained representing from 0.067 per cent to 0.1 per cent sugar. In case the solution is still lighter than the standard, after having added 1 c.c., this indicates that the blood contains less than 0.067 per cent sugar and so we add still another c.c. of the unknown solution to Tube 1 and by moving the piston again, we can obtain all shades representing from 0.033 per cent to 0.067 per cent sugar in the blood. In case there is more than 0.1 per cent sugar in the blood, the solution to be tested will be darker than the normal standard. In this case by moving the piston out we can obtain all necessary shades representing from 0.1 per cent to 0.2 per cent sugar. After pulling out the piston until we have removed 1 c.c. of fluid, if the liquid is still darker than the standard, it shows that there is more than 0.2 per cent sugar present,

and so we can use either a 0.2 per cent standard, or if we only want to use the 0.1 per cent standard, we push back the liquid into Tube 1 with the piston, and by means of a pipette withdraw 1 cc of liquid then add 1 cc of water, mix well with the pipette, and move piston in and out several times to insure proper dilution. Then by moving the piston out slowly we obtain all shades representing from 0.2 per cent to 0.4 per cent sugar, etc.

This colorimeter gives sufficiently accurate results for clinical work and can be made up very inexpensively to enable every physician to do any work requiring a colorimeter, regardless of whether he needs a colorimeter for one item or several.

310 WEST SEVENTY SECOND STREET

A GRADUATED TEST TUBE COLORIMETER*

BY ARTHUR T. BRICE, JR., B. A., FLORENCE, S. C.

THE instrument to be described was developed through the necessity of having available in the laboratory a suitable substitute for the standard instrument in the event that the latter should become accidentally damaged. Such an occurrence at a remote distance from optical repair facilities might easily have caused a disastrous tie-up of the clinical laboratory routine. A secondary motive was the possibility of offering to the young intern preparing to make a start in general practice an inexpensive item of equipment which might enable him to carry his knowledge of blood chemistry with him into his work, it having been our observation that the majority of young doctors refrain from installing the necessary equipment for this purpose solely on account of the expense of the colorimeter, which they estimate that they will have need for only occasionally. The necessary glassware and reagents for the most commonly employed blood chemistry determinations cost but a very few dollars, while the colorimeter which may be employed but once or twice a month will run close to a hundred and may not pay for itself in a very long time.

The instrument which we have evolved, and which we have called a Graduated Test Tube Colorimeter, was developed without any knowledge of the Denison Laboratories colorimeter, described by Peebles and Lewis,¹ and without any design to improve on this instrument, which, however, may be regarded as its direct lineal predecessor. It differs from the Denison instrument in several important features. The tubes of the Denison instrument being placed in a parallel position, it is impossible to view the colored solutions in both simultaneously, a factor of considerable importance for accurate and easy color matching. The ground glass diffusion screen employed in the Denison instrument also admits to the body of the instrument a wave front of

*From the Clinical Laboratory of the McLeod Infirmary, Florence, S. C.
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light of several times the square area of the cross section of the tubes themselves making extreme precision of construction necessary in order to satisfactorily eliminate disturbing reflected rays of light. In our instrument the tubes are aligned at an angle with each other making it easily possible to view the solutions in both simultaneously, and, the same bit being used for holding the tube barrels, two exactly equal pencils of light only are admitted to the body of the instrument. The work done with the Denison colorimeter, however, stands as a record of the reliability of the general principles underlying our instrument.

Myers has described an inexpensive test tube colorimeter employing the principle of dilution but this instrument, as he has himself subsequently pointed out,² suffers from several practical disadvantages, the principal one of which is that fairly large volumes of liquid are required in order to secure accuracy. The author believes that his instrument offers a more perfect optical system than Myers' side to side comparison, in that the whole volumes of

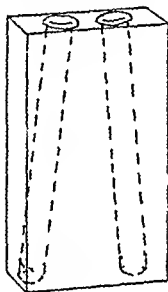


Fig 1

both liquids under comparison are under observation simultaneously, and no other volumes of the same or similarly colored liquids are in the field of vision.

The device consists of a block of wood through which two holes are bored to receive two graduated test tubes for the known standard and the unknown colored solutions which it is desired to compare. The block is preferably made from an inexpensive hardwood such as maple, though this is not necessary as soft woods are practically equally suitable. A convenient size is $32 \times 76 \times 165$ mm. Test tubes for such a block must be carefully matched so that columns of liquids of equal height will show identical readings by the graduation marks. This matching will usually be done with sufficient accuracy by the reliable supply houses if it is specifically stated in the order for what purpose the tubes are to be employed. We have found tubes of 15 ml capacity with minimum graduation interval of $1/5$ ml and an outside diameter of 13 mm, with the block described, to make a very compact and handy instrument.

In operation the instrument is held in one hand at a convenient reading distance from the eyes and sighted, preferably, but not necessarily, with both

eyes open, downward toward an evenly and brightly illuminated white surface such as a sheet of paper or a porcelain or enamel plate placed upon the table. A fixed setting may be employed, the liquid being poured into the tube to the desired mark and the tube then being placed in position in the block, or the liquid may be poured into the tube after it is in position in the block to any convenient height. The standard having been set in this manner, the instrument with both tubes in position is next sighted as described and the unknown solution poured into the other tube, a little at a time, sighting carefully after each addition, until an exact color match with the standard is obtained. Both tubes are then removed from the block and the readings made. One of the tubes may next be emptied and the matching process repeated any desired number of times to give a series of readings which are finally averaged and the calculation made according to the usual formulas for the standard prism types of instrument.



Fig. 2—Illustrating use of the instrument

The essential feature of design required to make the matching of colors easy by this method consists in properly aligning the bores of the test tube holes through the block, so that both tubes may be sighted through with one or both eyes open, and with a minimum of required movement of either the head or the block. If the alignment is correct so that an absolute minimum of motion is required, the faculties may be concentrated on the color comparison, and very accurate matching may be done. By practical experiment rather than from theoretical considerations the following dimensions have been determined. The distance between the tube bores from center to center at the top of the block should be 33 mm, and the central axes of the bores should form an angle with each other of 5 degrees. The axes should of course lie in the same plane. Using an instrument of these specifications, held at average reading distance of about 13 inches from the eyes, it has been found possible to match colors satisfactorily by operators of normal or corrected vision having interpupillary distances ranging from 62 to 66 mm. As interpupillary distances beyond these limits are rarely met with, it is felt that these specifications will be most suitable for the average.

The block is best bored accurately in a lathe setting it in a frame so that the bit will bore each hole at an angle of 25 degrees with the central longitudinal axis of the block. In order to permit the tubes to be readily inserted and removed the holes should be of inside diameter approximately 3 mm larger than the outside diameter of the tubes. The internal surfaces of the bores should be colored a dead black to minimize reflections and permit of only equal amounts of light to pass through the two tubes. This is easily done by dipping the whole block in a pan of dead black paint, or by pouring the paint through the bores carefully so that the entire internal surfaces are covered and then hanging the block to drain evenly and dry. The instrument can be manufactured at a total cost of less than two dollars.

In order to carry out a fair test of the accuracy of this instrument a series of 99 comparisons with the standard prism type instrument has been run. In performing these comparisons the determinations were first made accurately with the standard instrument, making two or three readings as is customary before the final calculation. A comparison by means of the graduated test tube colorimeter was then made, of the same two colored solutions, only making but one reading from which to calculate the value as determined by it. The series includes the following determinations: 2 spinal fluid sugar by Folin Wu technique; 3 blood iron by Wong technique; 3 blood urea nitrogen by Folin distillation technique; 4 blood performed creatinine by Folin technique; 20 blood nonprotein nitrogen by Folin micro Kjeldahl technique; 33 blood sugar by Folin micro technique; 34 blood sugar by Folin Wu technique. Assuming the determinations as made with the standard prism instrument to have been the true values the results of this series indicate the following factors of error. In 85 per cent of the determinations with the test tube instrument the error was less than 10 per cent. In 43 per cent of the determinations the error was less than 5 per cent. In 26 per cent of the determinations the error was less than 2 per cent. In 13 per cent of the determinations the error was less than 1 per cent. The maximum individual errors of 17 per cent and 18 per cent were found in the series in those procedures with which the technician was least familiar, spinal fluid sugar and blood urea nitrogen. These findings indicate that in the hands of the inexperienced a maximum error of as high as 18 per cent may be obtained but that with practice such as matching the standard against itself a number of times before each determination, and with care, such as averaging the readings of a number of comparisons before making the final calculation, sufficient skill may easily be acquired to make the use of this instrument entirely satisfactory to the general practitioner. The plan followed of making but one reading to arrive at the factors of error reported for the test tube instrument operated to its disadvantage, so that it is safe to say that these factors are high and that a considerably greater degree of accuracy would be found should the instrument be used only after practice and with care as prescribed. The mean average error of the graduated test tube colorimeter in the series reported was minus 1.542 per cent. The instrument is therefore offered

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A NEW TYPE OF MASK FOR THE STUDY OF RESPIRATORY EXCHANGE OF THE DOG*

BY ASHLEY W OUGHTERSON, M D,† NEW HAVEN, CONN

IN THE study of the respiratory exchange of the dog, one of the chief obstacles has been the development of an efficient mask. A satisfactory type of mask would be one in which the dead space was reduced to a minimum, that could be quickly and easily adjusted without discomfort to the dog and at the

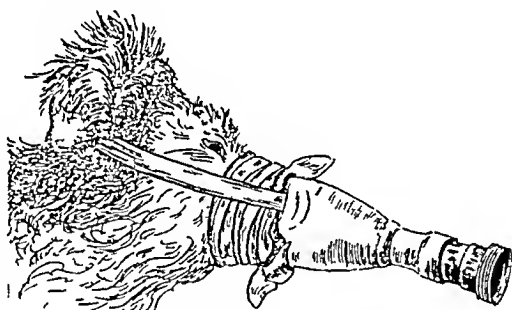


Fig 1—Mask adjusted to dog

same time would not leak. In the study of the basal metabolic rate the factor of making the dog comfortable is almost as important as the elimination of leaks.

Several types of masks have been devised by previous investigators. Among the earliest of these were those made of plaster of Paris for each individual animal according to the contour of the face. The mask was then covered with paraffin and an air-tight connection made by the use of plastacene or a similar material. This was difficult to hold in place and uncomfortable. Boothby and Sandiford described a mask which enclosed the entire head of the animal, the air-tight connection being made by means of wrapping the mask and neck of the animal with a strip of rubber dental dam 5 inches wide and 10 feet long. With this procedure it was necessary to shave the neck of

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the dog and it was difficult to adjust the wrapping around the neck with comfort. This type of mask was also used by Kitchen. The mask used by Kunde was simple and efficient although there was considerable dead space. It consisted of a metal cylinder across one end of which was stretched a sheet of rubber dental dam with a small hole in the center. A circumscribed area be

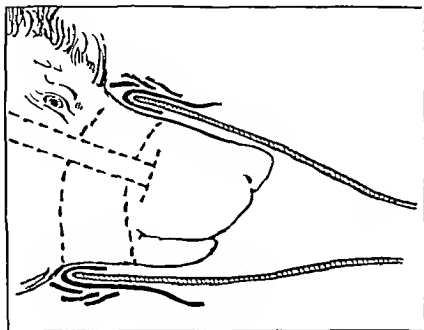


Fig 2—Shows principle of folded rubber contact.



Fig 3—Sheet and roll of rubber dental dam with mask

tween the corners of the mouth and the eyes was shaved and the muzzle of the animal inserted into the hole in the rubber dental dam. When widely varying types (e.g., short nose bulldogs) were studied, some difficulty was experienced in holding the mask in place. Blalock devised a rubber mask the rim of which could be inflated. The muzzle of the dog was first wrapped with a

strip of dental dam and the mask was then stretched over this and inflated. The mask was devised for use with the animal lying on its back and considerable difficulty was experienced in holding the mask in place with the animal on its side which position is necessary to obtain the basal metabolic rate. It was also not as comfortable to the dog as the one here described.

Fig 1 shows the mask fitted to the dog and Fig 2 shows the principle of the overlapping rubber to rubber contact. Fig 3 shows the sheet of rubber dental dam with a hole cut in the center. The size of the hole should be varied according to the dog so as to give a snug fit yet not be tight enough to cause edema of the tissue. It is not necessary to shave the muzzle, but a circumscribed area between the corners of the mouth and the eyes should be clipped with fine clippers. The sheet of rubber dam is drawn on over the muzzle and adjusted behind the corners of the mouth. The mask is then slipped on and secured with the straps behind the head. The edges of the rubber dam are then turned down and wrapped underneath the straps with a roll of rubber dam $1\frac{1}{2}$ inches wide and 3 feet long. The connection with the spirometer is made with an ordinary ground brass union. The straps are of assistance in holding the mask in place and are particularly useful during the training period. This mask has been used successfully in over five hundred determinations of the basal metabolic rate. From the illustrations, it will be seen that the dead space is reduced to a minimum. The mask is manufactured by the Baumann Rubber Company of New Haven, Connecticut.

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SIMPLE METHODS OF PRESERVING CULTURE MEDIA

BY HERMAN A. HEISE, M.D., UNIONTOWN, PA.

THE method of preserving culture media described here has been successfully employed in the laboratory of the Uniontown Hospital for the last ten years, and is recommended because of its simplicity and the fact that it requires neither ice box nor difficult technique.

The culture media is tubed and stoppered in the ordinary manner and the tubes then put into quart mason jars, fifteen to twenty of the ordinary



Fig 1—Method of arranging jars of Loeffler's blood serum in autoclave ready for sterilization



Fig 2—Completed culture media.

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sized tubes 120 by 16 mm being placed in each jar. Needless to say, jars of other sizes can be obtained to accommodate tubes of different measurements. If solid culture media is to be made the tubes are pressed against one side of the jar so that all lie parallel. The lid is screwed on loosely and the jars arranged in the autoclave, tilted at the proper angle for slants. The sterilization is performed in the usual manner except that the time is increased about 50 per cent, and the jars and autoclave are allowed to cool without having the contents disturbed. Not until the slants have cooled are the lids screwed on firmly and the jars may then be placed on shelves. This method is particularly applicable to the making of Loeffler's blood serum as smooth, moist slants free from bubbles are obtained, possibly because heating and cooling are delayed by the jars. It is very important, however, not to firmly fasten the lids tightly until the media has cooled, or bubbles will form.

In preparing liquid media for blood cultures the use of citrate bottles has been found most convenient. The media is placed in the bottles, the cover put on loosely, the spring catch not being pressed down in the locked position. A piece of cloth about six inches square is fastened over the head of the bottle with a rubber band and the media then sterilized in the autoclave. After sterilization has been completed the spring catch is pulled down sealing the bottle. Before use, the cloth covering of the bottle is soaked with an antiseptic to further guard against contamination.

ADVANTAGES OF THE METHOD

Culture media prepared in this manner remain sterile indefinitely, an ice box is not necessary, the media does not dry out, slants are uniform, and the technic is simple. No outlay of money for jars has been necessary at this institution, since these jars are continually brought to the laboratory as specimen containers.

AN IMPROVED PIPETTE MANIPULATOR*

By D C B DUFF, M A, VANCOUVER, B C

THE ordinary laboratory method of measuring out small quantities of bacterial antigens or other fluids in pipettes which are manipulated by mouth and hand are unsatisfactory for two reasons

1 The possibility of infection of the operator where live antigens are used is great, and may be pointed out as a possible source of the many laboratory infections by organisms such as *Br abortus*

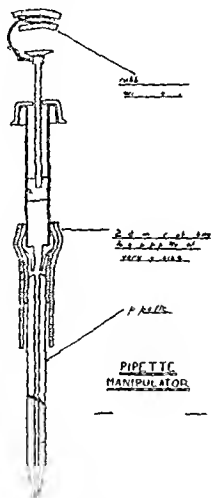


Fig 1

2 Even in the hands of experienced operators, accuracy in delivery by gravity is frequently sacrificed in the interests of speed

Carpenter, Boak, and Chapman¹ have described an instrument extensively used for this purpose on the continent, and known as a rheometer. This consists, essentially, of a graduated Luer syringe, to the plunger of which is attached an extension rod or piston. The plunger is normally kept in the "full" position by means of a coiled spring. The syringe is filled by pressing down the plunger and then allowing the fluid to be drawn up through a long needle

From the Department of Bacteriology The University of British Columbia
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Carpenter C M Boak R and Chapman O D J Immunol 17 73 74 1929

by the force of the spring. Delivery is made according to the graduations on the syringe by again pressing against the force of the spring.

Owing, however, to the fact that syringes are not as accurately graduated as are standard pipettes, and also to the fact that in the syringe the diameter of the meniscus is large, permitting errors in delivery, the rheometer offers certain disadvantages for work in which accuracy is at a premium.

A very simple alternative arrangement, which permits the use of standard pipettes, at the same time avoiding infections and allowing absolute accuracy, is here described. A Record type syringe, having a metal plunger of fairly stiff movement, is selected. Reference to the diagram explains the construction. A short length of heavy walled rubber tubing is slipped over the tip of the syringe, leaving a sufficient amount projecting to grasp and hold firmly the top of the pipette. The instrument may be adapted for use with pipettes of two different diameters by cementing in a shorter length of tubing of smaller diameter as shown, leaving at the same time a sufficient free length of the larger tubing to grasp the larger size pipette. A stiff wire is attached to the plunger and bent as shown. It is covered by a piece of tubing where the finger will come in contact with it.

In operation, a pipette is inserted into the rubber receiver. The barrel of the syringe is grasped with thumb and third finger, and the index finger is slipped between the plunger and wire guard. The finger may then be moved up or down, giving a positive manipulation both ways.

AN APPARATUS FOR THE SLOW INTRA-ARTERIAL INJECTION OF M/6 SODIUM CARBONATE SOLUTION AS AN ANTICOAGU- LANT IN BLOOD PRESSURE EXPERIMENTS BY MEANS OF COMPRESSED AIR*

BY WALTER E. GOWER, M.S., AND JOHN VAN DE ERVE, SR., M.D.,
CHARLESTON, S. C.

THE slow intra-arterial injection of one-sixth molar solution of sodium carbonate during the course of blood pressure experiments has been found very satisfactory for preventing clotting in the arterial cannula. In the Trendelenburg apparatus heretofore used the slow injection is accomplished by allowing mercury to drip slowly from a burette or mercury bulb into a stoppered flask which contains the solution and is connected with the manometer system (Fig. 1). The entrance of mercury into the closed system gradually displaces the fluid column in the tubing toward the cannula tip and the mercury flow is adjusted to keep the blood and anticoagulant mixing at that point. The same principle of fluid displacement by mercury has been used for the slow-timed intravenous injection of drugs.¹

*From the Laboratory of Physiology of the Medical College of the State of South Carolina.

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For general use in student laboratories the method has been found impractical because of the high cost of adequate amounts of mercury and the frequency of accidents with it in the hands of students. The simple arrangement described below eliminates the use of mercury but retains the advantages of the injection method.

In the apparatus, as diagrammed in Fig 2, a source of compressed air is used to force the solution from bottle *B* into bottle *A*. The rate at which the solution enters bottle *A* is regulated by adjusting the screw clamp *E* on the connecting tubing, and is observed by watching the rate of drop formation from the inlet tube in the air space at the top of bottle *A*. As only solution enters *A* the size of the air space remains constant except for slight changes with pressure fluctuations. Solution entering *A* raises the pressure and causes displacement of fluid from *A* into the manometer system.

The influence of the compressibility of the air in the air cap of bottle *A* on the movement of fluid in the manometer system can be eliminated by

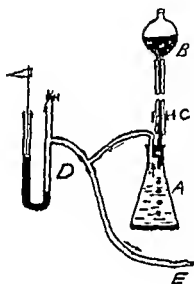


Fig 1

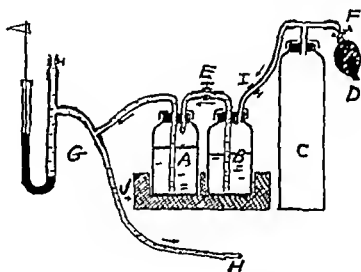


Fig 2

Fig 1—*A* Flask containing $M/6$ Na_2CO_3 , *B* Bulb containing mercury, *C* Screw clamp for regulating the rate at which mercury enters the flask, *D* Manometer system consisting of manometer and tubing, *E* Arterial cannula.

Fig 2—*A* and *B* Pressure bottles fitted with rubber stoppers which are held in place by screw caps, *C* Any source of compressed air, *A* bottle as represented may be filled with a hand bulb (*D*) after which the screw clamp *F* is closed, *E* Screw clamp regulating the flow of fluid from *B* to *A*. The rate of flow is observed by watching the drops fall through the air cap in *A*, *G* Manometer system, *H* Arterial cannula, *I* Screw clamp, *J* Wooden base for mounting bottles.

reducing the quantity of air to a minimum and by interposing a degree of relative resistance between *A* and the manometer system. Details for accomplishing this are shown in Fig 3 in which the inlet tube (*A*) is connected by means of rubber tubing with a burette funnel (*C*) through which a small glass tube (*D*) drawn to a fine point is inserted to make a water tight connection with the tubing about the funnel. With this addition air is forced from the tubing into the funnel followed by the fluid from bottle (*B*). The excess of air escapes to the top of the bottle and passes out through a special outlet tube governed by a screw clamp (*G*). The tip of the outlet tube (*F*) is fire polished to give a pinhole aperture.

If desired a simple manometer may be added to bottle *B* to indicate changes in the air pressure available. This is especially desirable when compressed air in a bottle is used as the source of pressure. A simple design

made from a heavy glass pipette is shown in the detail drawing of bottle *B* (Fig 4) Bottle *B* may be conveniently graduated to indicate the total amount of fluid injected

As pressure conditions exist in both bottles *A* and *B* some means must be devised to keep the stoppers in place We have found it convenient to use square histologic specimen bottles with a screw cap for both *A* and *B* The ones in use have a capacity of 140 c c The wide mouth takes a No 6 stopper, which is cut off to rest flush with the glass when in place The center of the screw cap is cut away to just clear the holes in the stopper When in place the screw cap overlaps the stopper at the margin and prevents it from being forced up by the pressure

If direct connection with a pressure line is not available a liter bottle filled with a hand bulb, or better, a tire pump is a very satisfactory source of

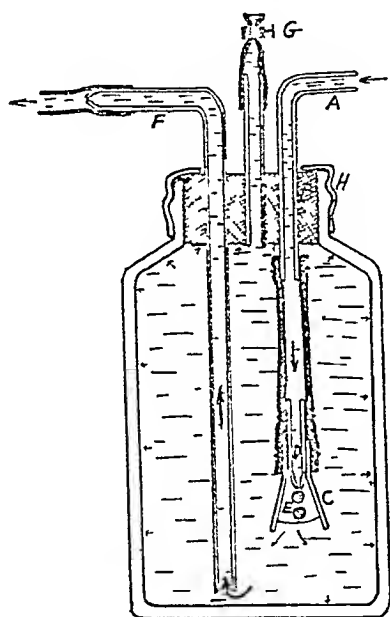


Fig 3

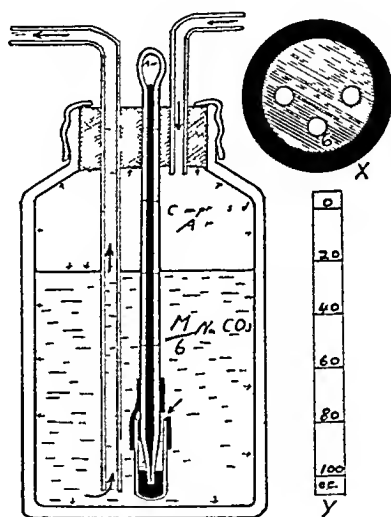


Fig 4

Fig 3—Modification of bottle *A* of Fig 2 *A* Inlet tube *B* Stopper held in place by the screw cap *H*, *C* Burette funnel *D* Small glass tube with dropper point *E* Air space in which the drops are observed *F* Outlet tube with tip fire polished to give a pinhole opening

Fig 4—Bottle *B* in Fig 2 with pressure gauge. A segment of a fine bore pipette is sealed in a flame at one end and the end expanded by air pressure while hot. The other end is held by the friction of a rubber connection in a small vessel containing mercury. The latter is made by annealing one end of a piece of glass tube in the flame until closed. An opening in the rubber is made as indicated to transmit the pressure to the mercury *X* Top view of stopper and screw cap *Y* Calibration of bottle.

pressure (*C* in Fig 2) One filling with reasonable pressure is more than sufficient to carry on a prolonged experiment displacing contents of bottle *B* several times

For convenient handling, bottles *A* and *B* are mounted together in a block of wood in which snugly fitting sockets have been cut

To use the apparatus, bottles *A* and *B* are filled to the desired level from a stock bottle by connecting a siphon tube with the glass delivery tube extending to the bottom of the bottles The displaced air escapes from the other

opening The apparatus is connected as described With the pinchcock between *A* and *B* closed, air pressure is admitted to *B* The pinchcock between *A* and *B* is then gradually opened, the air displaced from the tubes of the manometer system, the cannula tied in the artery, and pressure in the manometer raised to approximate the blood pressure expected The bulldog clamp, guarding the artery, is then removed and the fluid column allowed to oscillate with blood pressure fluctuations Fluid is admitted into *A* at a rate which just keeps the anticoagulant and the blood mixing in the cannula tip When bottle *B* is empty, the record of blood pressure need not be interrupted The screw clamp between *A* and *B* is tightened, the air supply is shut off, air is allowed to escape from the inlet of bottle *B*, and the bottle then disconnected and filled as before The bottle is then reconnected, the pressure restored, and the rate of admission into bottle *A* adjusted at the desired rate

By substituting a burette for bottle *B* and supplying air under pressure to the water column of the burette, the principle described is applicable to the slow injection of drugs under pressure at a measured rate For more rapid, and less accurately quantitative, injections the calibration of bottle *B* may suffice In either case the outlet tube from bottle *A* is connected by tubing directly with the needle used for the injection

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

CISTERN PUNCTURE Jacobi, L. Arch Dermat & Syph 19 651, 1929

The author favors the method described below

The patient may be sitting up or lying on one side, each position has its advantages and its drawbacks. The upright attitude offers clearer landmarks, but it requires an assistant to hold the head flexed and the use of a syringe to aspirate the fluid as there is usually no flow after a successful puncture, owing to negative pressure on the cistern. The recumbent position allows one to dispense with assistance and aspiration, the fluid flowing freely because of the positive pressure inside the punctured cavity. In spite of the somewhat greater difficulty of keeping the instrument in the median plane, the consensus of expert opinion favors the horizontal position.

Scrupulous asepsis of the needle, the hands and the field of operation is, of course, the first indispensable condition. Painting the nape of the neck with tincture of iodine is sufficient. The skin may afterward be removed with ammonia water or a solution of sodium thiosulphate.

The patient lies on the right side with the head moderately flexed and resting on a small hard pillow, which must be high enough to impart to the cervical spine a perfectly horizontal position. Moreover, the head itself should lie at right angles to the spine, or, as some express it, the cervical vertebrae, and the external occipital protuberance should form one straight line. Attention to these seemingly pedantic details may mean the difference between success and failure, since any considerable deviation from the "square" position will impart a wrong bias to the needle. Extreme flexion of the head is likewise to be avoided, as it tends to efface the osseous landmarks, though some degree of inclination is needed in order to put the atlanto-occipital membrane on the stretch.

With these preliminary requirements fulfilled, the operator is ready to proceed to the actual puncture. Placing the left index finger on the external occipital protuberance, he palpates downward in the middle line closely hugging the occiput, until the fingertip reaches the deepest depression. Immediately behind this spot will usually be found the spine of the axis, which is the first essential bony landmark. About 0.5 cm above it, the needle is inserted, pointing in the approximate direction of the outer end of the eyebrow (Spiegle).

No exact instructions can be given in this particular, as the proper direction of the needle will vary considerably with the degree of flexion of the head. For example, if the head is bent very slightly, the needle will have to be directed toward the eyebrow, on the other hand, with the head in extreme flexion and the chin resting on the chest, the needle will point proportionately higher. It will thus be readily seen that the imaginary prolongation of the instrument must describe an arc of several centimeters, corresponding to the degree of inclination of the head.

For this reason, Eskuchen himself does not specify any external landmarks for directing the needle, beyond the mere statement that it should be aimed at the place where one expects to find the end of the occiput. Warthenberg tries to be more explicit by giving the position of the instrument at the end of the operation. According to his observations, an imaginary prolongation of the needle, after it has reached the cistern, will pass between the eyebrows, or from 2 to 3 cm higher. He advises the operator to bear this terminal position in mind at the beginning of the puncture, and to be guided by it in choosing the right direction. When in doubt, he thinks it is better to aim a little higher, in order not to fall short of the occiput. Eskuchen also prefers to "reach the bone too soon rather than miss it altogether."

To the novice all these instructions may seem confusing but experience will soon enable him to find his way without difficulty

Puncture of the skin often meets with considerable resistance and should be made with a quick thrust, at the same time 'holding back' in order to avoid a sudden plunge forward. After passing the skin, the needle, advancing strictly in the middle line and in the direction just detailed, will soon encounter a bony obstacle which is the squamous portion of the occiput

The objective of the needle is the posterior edge of the foramen magnum. This second and extremely important bony landmark is called the point of orientation because it serves as a guidepost for the next and final phase in the entire procedure namely, the perforation of the fibrous atlanto occipital membrane which is all that now intervenes between the needle and the fluid in the cistern. Not always can this point on the edge of the foramen be reached at the first attempt. More frequently the instrument will impinge on the bony area above the edge, and it then becomes necessary to feel one's way along the occiput toward the foramen by tapping the bone lightly with the needle, after the manner of a blind man's staff, at each tap lifting the handle slightly and thus lowering the point. This manipulation soon brings the needle down to the foramen, and the exact place on its edge where the tapping instrument slips off the bone and meets the elastic resistance of the ligament below is the point of orientation. Here the atlanto occipital membrane is attached to the bone, and here the conditions for obtaining fluid are most favorable as the cistern is at its deepest about this level.

Should it happen that the needle in its first advance has fallen short of the occiput the point is raised by lowering the handle and the groping is continued upward until the bony edge is located.

The point of orientation is the crux of the entire Eskuehen method and it must be felt in every instance before proceeding further.

With the needle at the point of orientation, the operator raises the handle of the instrument in order to lower its point, removes the stylet and again advances very slowly and cautiously for a distance varying from 0.5 to 1.5 cm. The latter figure must not be exceeded, for it marks the outer limit of safety. Usually the lumen of the cistern is reached sooner sometimes after going forward a few millimeters. As the needle enters the cavity there is a characteristic muscular sensation of resistance suddenly giving way and almost immediately fluid appears at the hub. Sometimes this feeling of 'give' is absent hence the wisdom of removing the stylet in order not to 'overpass'.

SYPHILIS Delayed Darkfield Examination Mahoney J. F. and Bryant K. K. Ven Dis Inf 11 103 1930

A number of fine straight, glass capillary pipettes about 8 cm. in length and of constant bore are prepared.

The suspected lesion is abraded gently with gauze the resulting blood sponged away and the serum allowed to collect on the face of the lesion. The end of the capillary tube was then touched repeatedly to the lesion and the serum allowed to flow into the tube by capillarity. A mixture of 50 per cent vaseline and paraffin was used for the purpose of sealing the tubes. Press one end of the collection tube into a vaseline paraffin mixture until the plug at the opposite end is forced out and the serum collects in a drop at the upper end of the tube. The same objective can be accomplished by inserting a finely drawn capillary pipette into the lumen of the collection tube and aspirating the serum.

SPIROCHETA PALLIDA Staining of (Third Communication) Yamamoto T. Acta Dermat. Kyoto 14 145, 1929

In the first communication regarding staining of spirochetes the author found that 452 stains showed up spirochetes and he singled out 200 which yielded as good or better results as the twenty four hour Giemsa stain, for the three spirochetes named in the title. Testicular

syphilis and frambesia of rabbits was used on the height of infection. *Spirocheta cuniculi* was taken from condylomata near the rabbit anus. The smears were treated with a solution of ether and alcohol, equal parts, for fifteen minutes. All stains were used with 1 per cent watery or saturated solution, excepting in some instances a 5 per cent carbolic acid was added as a corrosive.

The dyes used in this study were methyl violet, 1713, which produces a deep violet color in *Spirocheta pallida* and *pallidula*, while it stains *Sp. cuniculi* violet blue. A few of the dyes, though of the same structure, produced other colors. Other stains used were baselin blue, erythrosine, neptune blue, acid blue, with carbolic acid, union violet, acid green, alkali blue, of various combinations with carbolic acid. Acid blue BBX with carbolic acid stains *Spirocheta pallida* and *pallidula* better than the twenty four hour Giemsa stain. *Sp. cuniculi* shows up negative under this same stain. Alkali blue always stains *Sp. pallidula* blue and *Sp. cuniculi* negative.

B TYPHOSUS New Medium For, Focosi, M. (Sull Utilita del Terreno al Ferrocianio-Tartrato Ferrico Potassico per l' Isolamento del Bacillo del Tifo) *Diagnostica e Tech. di Lab.* 1 246, 1930

The following solutions are required

- 1 Potassium ferritartrate 40 gm
Distilled water q s to make 200 cc
Dissolve in the cold and add 4 cc of liquid phenol
- 2 Lactose 250 gm
Distilled water q s to make 600 cc
- 3 Potassium ferrocyanide 40 gm
Distilled water q s to make 200 cc
- 4 Normal saline

The lactose and potassium cyanide are first dissolved, a small quantity of distilled water heated in a water bath and, when made to quantity indicated, are sterilized by heating to 100° C for thirty minutes.

The solutions are then mixed as follows

- Sol 1 180 cc
- Sol 2 430 cc
- Sol 4 40 cc
- Sol 3 175 cc

This stock solution may be kept indefinitely in a sterile well stoppered container. A greenish sediment may form and should be suspended by shaking before using the solution.

For use to 10 parts of the stock solution add 1 part of melted agar and after thorough mixing, pour plates. The reaction may be between P_H 7.2 and 7.8 but should not exceed 7.8.

Either pour or streak plates may be made.

B. coli gives azure colonies due to the formation of acid by fermentation of the lactose and the consequent production of Prussian blue.

B. typhosus colonies are yellowish white in color.

BLOOD SUGAR Accurate Determinations with One Tenth and Five Hundredths Cubic Centimeters of Blood, Pickard, R. J., and Pierce, L. F. *J. A. M. A.* 94 1134, 1930

The reagents used are tungstic acid solution, prepared by mixing 1 cc of 10 per cent sodium tungstate, 1 cc of two thirds normal sulphuric acid and 8 cc of distilled water, Folin Wu alkaline copper solution, and phosphomolybdic acid solution, which is discarded if it shows more than a pale color. A standard sugar solution containing 1 mg of dextrose per 10 c.c. is made from 1 g of dextrose solution preserved with toluene or xylene.

APPARATUS AND TECHNIC

The apparatus comprises a pipette measured to certain 0.10 and 0.05 cc for obtaining blood a pipette graduated at 0.1 cc (0.1 cc blood) and at 0.05 cc (for 0.05 cc blood specimen). Serologic pipette may be used if properly graduated. Heavy walled tubes 10 cm long with an inside diameter of 1 cm are used for centrifugation. Folin Wu sugar tubes graduated at 0.25 cc and at 3.12 cc and Ostwald Folin 0.10 and 0.25 cc pipettes are employed for the measurements.

A 0.1 cc portion of the tungstic acid solution is pipetted into the 10 by 1 cm tube. Into this 0.1 cc of blood obtained by ear puncture is expelled and washed and mixed by shaking when a chocolate color appears the tube is placed in the centrifuge and precipitated at high speed (For 0.05 cc of blood 0.9 cc of tungstic acid solution is used). The usual Folin Wu technic is then followed 0.5 cc of the supernatant fluid being pipetted into a Folin sugar tube with a 0.5 cc Ostwald pipette. 0.5 cc of the standard into another tube 0.5 cc of the alkaline copper solution added to each and both placed in boiling water for six minutes. They are then cooled 0.5 cc phosphomolybdic solution is added to both the standard and the blood the solution is allowed to stand five minutes to develop the color diluted to the mark mixed and read in the colorimeter. If the unknown is set at 20 mm in the colorimeter and matched against the standard the reading of the standard multiplied by ten is the sugar content in milligrams per hundred cubic centimeters of blood. For example if the unknown is set at 20 and the standard matches at 10.2 the blood sugar is 102 mg per hundred cubic centimeters of blood. Readings should be recorded to the nearest 5 mg.

The greater error inherent in the Folin Wu method with high dextrose readings is the reason for the dilution of the blood to 1:20. The tungstic acid solution contains double the proportion of acid ordinarily used with 0.10 cc of blood this gives a more rapid precipitation and a more concentrated precipitate by centrifugation with a resultant larger amount of clear supernatant fluid. Rechecks can be made without difficulty on the same specimen.

MALARIA Thick Film Method Barber M. A. and Komp W. H. W. Pub. Health Rep. 44:2330 1929.

The authors insist on the importance of a good quality Giemsa stain and that the water used for diluting it should be neutral or slightly alkaline and free from salts. They employ Grubler's Azur Eosin solution which can be obtained from Karl Holborn of Leipzig. The distilled water should have a reaction of P_H 7.0 to 7.2. The blood can be conveniently collected from a puncture made on the dorsum of the middle finger a little below the base of the nail care being taken not to touch the patient's skin with the slide which must be free from grease. A drop of blood about three or four times as much as is required for a thin film is taken onto the slide near one end and is spread out with the pricking needle over an area the size of the little finger nail. The patient's number is written on the other end with a green pencil. The slide is placed film side downward in its groove in the slide box which is stood on its end until the blood is dry enough not to run. Slides kept overnight in a closed box are sufficiently dry for staining next morning. Alternatively the box may be left without its lid in the incubator for sixty to seventy five minutes. If drying is insufficient the film will come off in the staining bath if it is too prolonged the parasites will not stain properly. If it is necessary to keep the slides for some time before they can be stained excessive drying can be prevented by wrapping them in paraffined (not kerosined) paper. The authors find that preliminary dehemoglobinization and fixation of thick films are unnecessary. Sufficient stain for 25 slides is prepared by putting 60 or 70 drops (13 cc) of Giemsa solution in the staining dish and adding 75 cc of water. The slides are left in the stain for about half an hour. Differentiation is obtained by placing them in distilled water for five minutes. If the background is deep blue and the leucocytes almost black the preparation is overstained. Thin films may be made on the same slide as the thick films at the other end and the labeling done with an ordinary lead pencil on the thin film. The thin film can be stained later with Leishman's stain if it is necessary to

determine the type of parasite, a line drawn across the slide with a grease pencil prevents the stain running on to the thick film. It is unsafe to call anything a parasite unless it shows a red dot of chromatin associated with a blue mass of cytoplasm. Stained films can be preserved by covering them with liquid paraffin, or vaseline, and storing away from light. Immersion oil must first be removed with xylol, after warming the slide slightly, and the xylol removed with absolute alcohol. When large numbers of slides require staining, small pieces of cardboard can be placed between the numbered ends, and the slides held in place by a rubber band. The block of slides can then be placed upright in the staining dish, with sufficient staining solution to cover the thick films at the other end.

TISSUE Rapid Diagnosis of Malignant Tumors, Dengler, R. Zentralbl f Gynak 53 457, 1929

A rice seed sized piece of tissue is removed from the suspected part and dissected with the help of two needles in 0.9 per cent NaCl solution. It is examined with high magnification and almost closed diaphragm after the addition of a drop of 1 per cent acetic acid. The nuclei of the tumor cells are counted according to their size. The smallest nuclei present are counted with 1, the nuclei twice the size with 2, etc. to 7. The result is expressed as a quotient of the number of different sizes counted divided by the total number of nuclei counted. If fifty nuclei are counted, the quotient for carcinomas is always above 0.1, usually 0.14. The range, in general, is from 0.06 to 0.14 (from 3/50 to 7/50). The diagnoses were made from fresh tissue in 100 cases and later substantiated from sections prepared with the usual methods.

B TYPHOSUS A New Method of Isolation from Feces, Tifico, B. Bull d Sez Ital d Soc Intern di Microbiol 1 275, 1929

The author adds to the fecal suspension typhoid agglutinating serum and, after standing, centrifuges.

The sediment is washed with normal saline, a new dose of agglutinating serum added and again centrifuged. It is then plated.

STREPTOCOCCI From Scarlet Fever, Erysipelas, and Septic Sore Throat, Tunncliffe, R. J A M A 94 1213, 1930

Tunncliffe reports that hemolytic streptococci from typical cases of erysipelas produce a bright green on chocolate agar after from twenty-four to forty-eight hours' growth, while those from scarlet fever cause no change or occasionally a slight greening of the medium after several days' growth.

Immunologically (opsonic method), hemolytic streptococci from septic sore throat belong to neither the scarlet fever nor the erysipelas groups. They differ from scarlet fever streptococci by causing chocolate agar to become green.

On chocolate agar, typical colonies of streptococci from scarlet fever are slightly granular and conical, and from erysipelas smooth and convex, while those from septic sore throat are very rough, indented, conical, or convex, and gunmetal in color, with occasional blister-like tops.

INDICANEMIA Value of in Cases of Renal Insufficiency, Monias, B. L., and Shapiro, P. Arch Int Med 45 573, 1930

The following method is described:

Either oxalated plasma or serum may be used. Plasma has the advantage that one need not wait for the clot to separate from the serum, and in that it can be taken from the samples already collected for the determination of the nonprotein nitrogen, urea nitrogen and creatinine. However, it is essential to use lithium oxalate, since potassium oxalate seems to interfere with the color reaction (as it does also in the determination of uric acid).

Serum has the advantage that no special lithium oxalate bottles are required. The blood sample may be placed in any available tube.

The following reagents are required: (1) 20 per cent trichloroacetic acid, (2) 5 per cent alcoholic solution of thymol, (3) concentrated fuming hydrochloric acid (chemically pure, specific gravity 1.19), containing 0.5 per cent ferric chloride, (4) chloroform (Merek's reagent, chemically pure) and (5) distilled water.

The following apparatus is required: (1) a 100 cc graduated cylinder, preferably with glass stopper, (2) an Erlenmeyer flask of about a 200 cc capacity, and (3) a filter funnel.

Quantities may be measured either gravimetrically or volumetrically. Five grams or cubic centimeters of the separated plasma or serum are placed in an Erlenmeyer flask. An equal amount of distilled water is added. The mixture is precipitated by adding an equal quantity, i.e., 10 gm or cc of the trichloroacetic acid. The flask is then rotated for about two minutes. After five minutes the contents are filtered into the graduated cylinder. Through a capillary pipette, 10 drops of the thymol solution are added to the clear filtrate. Then is added an amount of the hydrochloric acid ferric chloride solution equal to that of the filtrate obtained. The mixture is shaken well and allowed to stand for two hours. Then 5 cc of chloroform is added, and the mixture is shaken for two or three minutes and allowed to stand. The chloroform dissolves out the indigo and promptly settles to the bottom.

For accurate, quantitative readings, the chloroform is pipetted off, and the intensity of its coloration is compared with an artificial or natural standard solution. A Duboseq or Autenrieth colorimeter is used for this purpose.

Preparation of the Natural Standard. Three milligrams of indican are dissolved in 30 cc. of chloroform and placed in a graduated glass cylinder. To this solution are added 30 cc. of a 0.5 per cent solution of ferric chloride in concentrated hydrochloric acid, and 30 capillary drops of a 5 per cent alcoholic thymol solution. The mixture is shaken well and left standing for two hours. The colored chloroform is pipetted off, and the remaining solution is shaken with another 10 cc. of chloroform which is added to the portion previously removed. This procedure is repeated until the total amount of chloroform is 60 cc. This 60 cc. contains 3 mg. of indican. 10 cc. contains 0.5 mg., or 100 cc. contains 5 mg. By using this chloroform solution as the standard the calculation of the unknown quantity is done as follows:

$$\frac{\text{Reading of the standard} \times 0.5}{\text{Reading of the unknown} \times 10} = \text{milligrams of indican per 100 cubic centimeters of blood}$$

If the coloration of the standard is too intensive, further dilutions may be made with chloroform. Of course, these dilutions are taken into consideration in calculating the unknown quantity.

Preparation of the Artificial Standard. Indican cannot be obtained on the market. It is cumbersome to prepare and requires special technical skill and laboratory facilities. The necessity of preparing an artificial standard has been recognized by earlier investigators. Thus, Erick suggested a mixture of scarlet red and eriochrome (Gruebler). For the artificial standard the authors selected two dyes which are found in every laboratory, namely, eosin (water soluble, yellow) and gentian violet. The artificial standard consists of 6 capillary drops of a 1 per cent solution of gentian violet in water, 3 drops of a 1 per cent solution of eosin in water, 10 cc. of 94 per cent alcohol and 30 cc. of distilled water. This mixture corresponds in tint and intensity to the original standard of 5 mg. of indican in 100 cc. of chloroform. From the stock solution, various dilutions may be made, water being used as a diluent. Besides being easily obtainable, this artificial standard has the advantage of not fading. The higher dilutions of the natural standard fade with time.

To facilitate the dilutions of the standards the accompanying tables are given.

TABLE I (STANDARD 1)

DILUTIONS OF STANDARD SOLUTIONS WITH EQUIVALENTS OF INDICAN

AMOUNT OF STOCK SOLUTION CC*	AMOUNT OF CHLOROPHYLL ADDED CC	EQUIVALENT OF INDICAN MG †	INDEX OF INTENSITY OF INDICAN
10	0	0.5	
9	1	0.45	4 plus
8	2	0.40	
7	3	0.35	
6	4	0.30	3 plus
5	5	0.25	
4	6	0.20	
3	7	0.15	2 plus
2	8	0.10	

*Stock solutions of smaller amounts may be used
 †One hundred cubic centimeters of the stock solution corresponds to 5 mg of indican

TABLE II (STANDARD 2)

DILUTIONS OF STANDARD SOLUTIONS WITH EQUIVALENTS OF INDICAN

AMOUNT OF STOCK SOLUTION CC*	AMOUNT OF CHLOROPHYLL ADDED CC	EQUIVALENT OF INDICAN MG †	INDEX OF INTENSITY OF INDICAN
10	0	0.05	
9	1	0.045	1 plus
8	2	0.40	
7	3	0.035	
6	4	0.030	Trace
5	5	0.025	
4	6	0.020	
3	7	0.015	
2	8	0.010	Negative
1	9	0.005	

*Stock solutions of smaller amounts may be used
 †Artificial stock standard (10 cc or 0.5 mg of indican) in the same way as the natural standard solutions being used as a diluent

†One hundred cubic centimeters of the stock solution corresponds to 0.5 mg of indican

CEREBROSPINAL FLUID New Method for Estimation of Albumin in, Arnaud, R Bull Soc path exot 22 337, 1929

The method recommended by the author consists in the precipitation of albumin at ordinary temperatures by the following reagents:

Acetic acid	50 cc
Carbon tetrachloride	15 cc
Alcohol, 95 per cent	240 cc

The mixture should be well shaken and preserved in a colored and stoppered bottle. The technic consists in pouring 4 cc of the cerebrospinal fluid into a Sieard's tube and adding at least 1 cc of the reagent. At the zone of contact of the two fluids there is a milky precipitate, the tube should then be stoppered and the two fluids well mixed. The results should be read after twenty minutes in the usual way.

It is claimed that the results are comparable with those yielded by the classical procedure, that the precipitate is even more regular and consequently the reading easier, and that it saves at least four and one-half hours.

Sice and Boisseau (Bull Soc path exot 22 679, 1929) report as follows on this method. In the case of normal spinal fluids and those containing but slight excess of albumin the differences, although marked, are not of great significance, but as the quantity of albumin increases so do the differences in values obtained.

The conclusion reached is that Arnaud's new method does not constitute any advance and should not replace the classical method of employing hot trichloroacetic acid for five hours

B DIPHTHERIAE The Stoltenberg Stain Owen H H. and Band M. *Am Jour Pub Health* 20 426 1030

The formula and procedure for Stoltenberg's stain are as follows

Malachite green	0.250 gm
Toluidin blue	0.050 gm
Hematoxylin or logwood extract	0.010 gm
Distilled water	100.0 cc
Acetic acid	3.0 cc
Ethyl alcohol	3.0 cc

Stain for 1 minute. Then wash the preparations with tap water and dry. The granules appear red and the body green.

The stain is not recommended as a substitute for Loeffler's alkaline methylene blue stain for routine use as it does not so satisfactorily differentiate *B. diphtheriae* from *B. xerosis* and other nonpathogenic forms with polar staining. It is recommended as a valuable confirmatory stain however since it aids in the differentiation of *B. diphtheriae* from *B. hofmanni*, especially in young cultures and is a very short and simple procedure.

SPIROCHETES Silver Starch-Gelatin Method for Tissues Warthin A S. *Brit J Ven Dis* 5 255 1029

A. For material well fixed shortly after removal from body

1. Remove paraffin from cover glass preparation pass through alcohol and water and place in the oven in about 10 cc of 1 per cent nitric acid for thirty minutes.

2. Wash in distilled water for ten to fifteen seconds and then continue with the modified Warthin Starry method.

B. For material poorly fixed or showing postmortem changes

1. Remove paraffin from cover glass preparation pass through alcohol and water and place in 1 per cent nitric acid for one minute.

2. Wash in distilled water for ten to fifteen seconds and continue with the modified Warthin Starry method.

In the case of old tissues much over fixed in alcohol the following routine may be used

1. Remove the paraffin from the cover glass preparation pass through alcohol and water, and place in nitric acid of 2 to 10 per cent strength for from fifteen to sixty minutes. The greater the acid concentration the shorter the period of immersion in the acid necessary.

2. Wash in distilled water from fifteen seconds to three minutes. The stronger the acid used the longer should be the period of washing.

3. Dip in 2 per cent silver nitrate and proceed with the starch gelatin method. Longer development may be necessary with the increased acidity of the section but there results a lighter background with heavily stained organisms and a cover glass almost free from precipitates.

This method of 'freshening' the tissue has been used with great success in stock control tissue that has been kept in the laboratory for over twenty years in which it was becoming increasingly difficult to obtain well stained spirochetes. Hydrogen peroxide had been used with varying degrees of success but at the best was uncertain. By the use of nitric acid as above directed beautiful preparations are now easily obtained with this old material. The spirochetes appear intensely black on an almost colorless background. Other material which had been preserved under similar conditions but not for so long a period gave even better results. The sections dried on the cover glass without the use of albumin fixative, become detached when the stronger acid solutions are used for an hour or longer at a temperature of 30 to 40 C. On the other hand in dilutions too great the tissues are not sufficiently acted upon by the acid to produce the proper result. In fresh cases fixed for not more than several days and then embedded in paraffin the acid treatment should not be

prolonged in case the fixation was poor or the spirochete on the point of breaking up when fixed, since the action of the acid tends to break up the spirals and give the beaded effect of degenerating spirochetes. In such tissues the sections may be dipped in dilute acid for one minute, and a favorable tissue reaction thus established.

Directions for using the Starch Gelatine Modification of the Warthin Starry Method of Staining *Spirocheta pallida* in Single Tissue Sections on the Cover Glass, with the Preliminary Nitric Acid Treatment

- 1 The tissues should be well fixed in formal, larger pieces requiring more time than smaller ones
- 2 Transfer blocks of convenient size to 96 per cent alcohol for one hour or longer. Follow by three changes of absolute ethyl alcohol for one hour each at a temperature 50° to 55° C
- 3 Run through two changes of xylol for half an hour and an hour respectively at room temperature
- 4 Press the xylol out of the tissues on filter paper and pass through two changes of paraffin for half an hour and eight to twelve hours each at 50° to 55° C
- 5 Block in paraffin
- 6 Cut sections 6 to 10 microns thick and transfer on to water just warm enough to flatten the section without melting the paraffin at about 35° to 40° C. Distilled water free from bacteria must be used, or there will be a precipitate of silver between the tissue and the cover glass
- 7 The perfectly clean cover slip is then immersed in the water perpendicularly at the edge of the floating section and then lifted out with the section on it. If the glass is wholly free from any contaminations the tissue will adhere to it. No albumin fixative is used. Number 1 cover slips of suitable dimensions are employed, and with a little practice the sections can be readily centered
- 8 The cover glass preparations are then allowed to dry for two hours at 55° C or overnight at 35° C
- 9 The paraffin is removed from the section by flaring slightly and putting through two changes of xylol, two changes of absolute alcohol, 96 per cent alcohol and water
- 10 The cover glass preparation may now be treated with nitric acid solution as described above or immersed directly in 2 per cent silver nitrate, and placed face down upon another perfectly clean cover slip (the tissue then being between the two cover slips), and the two cover glasses adherent by capillary attraction are put upright at the edge of a clean bottle containing sufficient silver nitrate solution to cover the lower half of the two cover slips only. When nitric acid is used the section must be washed as directed in distilled water before it is put into the silver
- 11 The incubation in the silver nitrate solution should be carried out in a dark oven at 50° to 55° C for from thirty minutes to two hours, depending on the type of tissue involved. A dense fibrous and elastic section, such as aorta, requires longer impregnation than does one of liver or lymph node. Fetal tissues and those treated with nitric acid require less exposure to silver nitrate than do those of adults, especially if overfixed
- 12 The opposed cover-slips with the section between them are taken from the silver solution and the cover glass forceps slipped between them, prying them apart. It is unwise to slide the glasses apart, as the tissue may be injured or partially loosened from the cover to which it is adherent. The cover glass with the tissue is placed section side uppermost in a watch glass or other flat container, and the developing mixture, consisting of 1 part of 2 per cent silver nitrate added to the starch gelatine acetone quinol 5 parts (see below), is poured over it, so that a layer at least 3 mm thick is over the tissue
- The reduction is allowed to proceed until the section is of a medium yellow or pale brown appearance
- 13 The section is then removed from the developer and washed for a few seconds in warm water to ensure removal of the starch and gelatine

14 It is then passed through a 5 per cent sodium hyposulphite solution to remove any remaining silver nitrate

15 Wash in distilled water, 96 per cent alcohol two changes of absolute alcohol two changes of xylol and mount in balsam

DEVELOPING SOLUTION

The developing solution is made up as follows

1 Ten grams of gelatine are dissolved in 100 cc of distilled water using a double boiler to prevent burning

Strain while hot through a clean cloth into the stock bottle

2 Ten grams of starch (Argo or Kingsford's) are mixed with a few cc of cold distilled water to make a thick paste, and then 100 cc of boiling distilled water are added. This is stirred and then poured into the hot gelatine in the stock bottle without straining

3 Five cc of acetone are dissolved with 7 cc of a fresh 5 per cent solution of quinol and are added to the starch and gelatine in the stock bottle, which is immediately tightly stoppered and vigorously shaken.

4 This mixture is allowed to cool when it will become solid. Whenever it is to be used it is placed in the oven and warmed until liquid

5 Just before the developer is poured over the section a 2 per cent silver nitrate solution is added in the proportion of 1 to 5 and well mixed by pouring from one beaker into another several times

The fewer the sections developed at one time the better the results but any number may be done, as long as there is sufficient of the developer to cover all to the uniform depth of 3 to 5 mm. In this laboratory never more than eight sections are developed at once

6 If the stock developer is allowed to stand in the melted condition for very long at a time it will settle to the bottom, in which case a vigorous shake will restore it to its original condition. It is unnecessary to add any preservative to prevent bacterial decomposition

BLOOD GROUPS Determination of in Cadavers Holzer F J Klin Wchnschr 8 2427, 1929

Holzer points out that the determination of the blood group in cadavers is frequently difficult because of the plasma of the blood which is not sufficiently clear to permit demonstration of the agglutinins. It is known that the agglutinins may also be found in other body fluids. In cadavers the pericardial fluid remains free from blood pigments and from putrefactive bacteria longer than other fluids, consequently it was considered the best material for the determination of the blood groups of cadavers. The author states that this method was employed in a large number of corpses and it proved particularly valuable in determining the blood group of cadavers of infants and of fetuses

BLOOD PARASITES Demonstration of in Peripheral Blood of Kala-Azar Shortt H E Das S and Lal C Indian Jour Med Res 15 529, 1927

A small drop of blood is placed at one end of a slide a second slide is applied to it, as in making an ordinary blood smear. The second slide as soon as the blood has spread out along its edge, is pushed along the surface of the first with an even motion until the blood is almost exhausted. At this point instead of continuing this motion as in making an ordinary smear the slide is abruptly lifted off with the result that the blood smear ends in a straight edge stretching transversely across the slide. This straight edge is somewhat thicker than the rest of the smear and contains a large percentage of the total white cell content of the drop of blood. The white cells in the straight edge are all that it is necessary to examine for the purpose of determining with a fair degree of accuracy, the presence and numbers of Leishman Donovan bodies in the peripheral blood

RETICULOCYTE COUNT In Normal and Abnormal Conditions, Friedlander, A., and Widemeyer, C Arch Int Med 44 210, 1929

The following method, for which great accuracy is claimed, is used by the authors

A specific pipette for the making of a blood dilution of 1 20, instead of the usual white cell pipette (dilution 1 10) is used

A microscopic objective, giving a magnification higher than the ordinary 4 mm lens, is of advantage The authors have used a No 7 Leitz or a DD Zeiss The oil immersion lens cannot be used

The solutions needed are

1 A 1 per cent aqueous solution of brilliant cresyl blue We have used the Gruebler, natural aniline and Coleman Bell dyes The Coleman Bell dye has given the best results

2 A diluent solution containing 0.6 gm of sodium chloride and 0.2 gm of potassium oxalate to 100 cc of distilled water

These are the stock solutions They can be kept indefinitely in a cool place As needed, another dilute solution is made up as follows

Two cubic centimeters of the brilliant cresyl blue solution (solution 1) is added to 8 cc of the diluent solution (solution 2) which makes a 0.2 per cent solution of brilliant cresyl blue This solution (solution 3) must be made up fresh about once a week It is necessary to vary the dilution from time to time, depending on the dye used

The blood is drawn up to the 0.5 mark of the pipette with a dilution of 1 20, then it is diluted to the 21 mark with the 0.2 per cent brilliant cresyl blue solution (solution 3) The pipette is shaken thoroughly, allowed to stand for ten minutes or more and then shaken well again The blood is then ready for counting The ordinary counting chamber is used, and the squares are counted as they are in the ordinary white cell count In calculating the result it must be remembered that the dilution is twice that of the ordinary white cell count The blood, diluted in this way, may stand twenty four hours without injury to the cells, but after this period the count is not accurate To count the reticulocytes, it is necessary to have a good light, preferably artificial, and an especially high magnification dry lens as before mentioned With this dilution of stain, the white blood cells stain dark blue The red cells have a pale yellow cast, but show up blue The reticulocytes are easily recognized by the blue reticulum or granules in their cytoplasm The various forms of reticulocytes, as enumerated by Seydewitz, are brought out distinctly These vary from the earliest forms, with dense reticulum in the center of the cells, so that they resemble normoblasts, to the more mature forms showing only a few scattered basophilic granules

When normoblasts or megaloblasts are present, there is a distinct difference in staining reaction between the nuclear and reticular material The nuclear material stains purple, the reticular substance stains blue

This method overcomes the chief objections to the Cunningham method which is the method in general use In the Cunningham method the stain does not come in contact with the cells of the blood droplet, and the reticulocytes are not evenly distributed over the slide It is much simpler than counting 1,000 red cells in a stained preparation As a matter of fact, by this method a reticulocyte count can be done as easily, and almost as quickly, as a white cell count after some experience has been had with the method

TUBERCULOSIS A Cholesterol Agglutination Reaction, Hinton, W A., and Stuart, G O N E J Med 202 327, 1930

The dried and killed growth of three strains of tubercle bacilli, after being kept in stoppered glass bottles for approximately four months, were treated as follows

One part by weight of the bacillary mass is extracted with eight volumes of ether, during the extraction shaking for ten minutes This extraction is repeated four times

The ether insoluble bacillary residue remaining is then extracted for three days at a temperature with five volumes of 95 per cent alcohol, shaking vigorously several times

For the conduct of the test the "glycerinated indicator" is prepared each time as follows

To 1 part of alcoholic extract ether insoluble biliary residue add 9 parts of 0.7 per cent alcoholic solution of cholesterol

To 1 part of this mixture add 2 parts of 5 per cent aqueous solution of sodium chloride and immediately shake vigorously. Then add a further 12 parts of 5 per cent sodium chloride aqueous solution and again shake. Finally add 15 parts of 50 per cent neutral glycerin and again shake vigorously

The test

Place 0.1, 0.3 and 0.5 cc of serum to be tested in each of three agglutination tubes and to each add 0.5 cc of glycerinated indicator. Shake well and incubate at 37° C overnight

A positive reaction produces definite agglutination of cholesterol particles thus clearing the fluid

RETICULOCYTES Determination of Holboell, S. A. Ugeskr. f. Læger 91 1077, 1929

The following technique has been used in the author's department with satisfactory results. Place in a small receptacle 25 cc of 1 per cent brilliant cresyl blue in a 0.9 sodium chloride solution (the pipette may be utilized which serves for the counting of white blood corpuscles). To this add 0.1 cc blood (taken for example with the pipette which serves for the blood sugar determination). After careful mixture of the blood and the staining fluid the smear preparation after half a minute is ready for the cover slip, etc.

When the specimen has been dried in the air it is fixed for three minutes in pure absolute methyl alcohol. Under this treatment the color will disappear except in the vital stainable substance. The methyl alcohol is poured off and the usual Giemsa stain is applied, treating the specimen with a freshly prepared dilution of the Giemsa solution for Romanowsky stains (Gruebner and Co.) 15 drops per 10 cc of distilled water. After fifteen minutes it is washed with distilled water and the specimen is dried with several layers of filter paper. Specimens prepared in this manner extremely rapidly show the existence of reticulocytes. The vital stained substance is colored blue and differs sharply from the pale red fundamental color of the red blood corpuscles.

UREMIA The Diazo Color Reaction in Rubinowitch I. M. Arch. Int. Med. 45 282, 1930

A positive diazo color reaction is not found in any condition other than severe kidney damage.

A positive reaction may frequently be found in persons with severe kidney damage and such persons may recover. Such cases include the albuminurias of severe infections the well recognized form of acute nephritis with pallor edema etc. acute exacerbations of chronic nephritis mechanical obstruction to the urinary flow, surgical lesion of the kidneys with urinary retention and the anuria of diabetic coma.

When acute exacerbations of the disease are excluded a positive reaction occurring during the course of chronic nephritis has invariably at least in our experience meant unfavorable prognosis.

When all of the aforementioned factors are given consideration the test is of value in differentiating uremia from cerebral arteriosclerosis.

BACTERIOLOGY Detection of Ammonia Production in Agar Slants, Hansen P. A. J. Bacteriol. 19 223, 1930

1 cc of a solution of thymol and 1 cc of a hypobromite solution are added successively to the culture and allowed to act for twenty minutes. If ammonia is present, the mixture becomes blue or greenish blue. The blue color may be extracted by means of ether in which it is soluble, resulting in a deep red violet color. The reaction is also given by certain aliphatic amines and by glycine. It has the advantage over the Thomas test of not

using a hypochlorite The hypobromite is more easily prepared fresh and of definite strength each time it is used This is done by mixing bromine water with sodium hydroxide Like the Thomas test, only weak solutions give the ammonia reaction

SPINAL FLUID Bicolored Guaiac Test, Latham, O Med J Australia 802, Nov 30

The reagents

1 British Pharmacopoeia tincture of guaiacum resin, 20 per cent in alcohol sample freshly made up can be got from any chemist

2 Brilliant (diamant) fuchsin, 0.5 per cent in absolute alcohol

3 Naphthol green, 0.5 per cent in distilled water

4 10 per cent sodium chloride from which we make the 0.2 per cent saline the spinal fluid dilution

5 A 0.5 per cent solution of anhydrous sodium carbonate

Keep all in dark glass stoppered bottles in a cool place

Preparing the colloidal solution

Place 40 cc of distilled water in a small Erlenmeyer flask, then pour side of flask with pipette, 0.22 cc of guaiac tincture in 9 cc of absolute alcohol added), lightly and continuously shaking the flask The suspension should be white, transparent and only feebly opaque to overhead light Still agitated in circular manner add 2 cc of naphthol green solution As soon as the mixture turns slightly pink add 0.3 cc of the diamant fuchsin solution The result should be a cherry slightly opaque mixture to overhead light Some samples appear purple but seem to act After twenty minutes it is ready for use

The test The usual series of dilutions are made in ten small test tubes $\frac{1}{2}$ cc of spinal fluid is placed in the first two tubes Then still in the first tube, add to each tube $\frac{1}{2}$ cc of 0.2 per cent saline, containing 100 cc of sodium carbonate to each 100 cc of saline The saline mixture should be the strong saline solution Beginning from the second tube, transfer $\frac{1}{2}$ cc and after mixing $\frac{1}{2}$ cc to the fourth and so on, as is usual with serial dilutions each tube then add $\frac{1}{2}$ cc of the colloidal suspension Gently shake

Results No change—0, the solution remains unchanged, red light red color with slight precipitate, 2, greyish red color with slight greyish green color, red precipitate, 4, vivid green color with red precipitate Read after twenty to twenty four hours A typical paratyphoid 4443321000 as in the gold solution test

ANEROBES A Simple Method for Petri Dish Cultures, Herrington

J Bacteriol 19 101, 1930

The petri dishes are poured as for aerobic cultures, except that agar, not exceeding 8 cc, is used The plates are allowed to set in a vacuum desiccator over a dish containing water at about 100°C placed directly at the bottom of the desiccator It is desirable that the cover be warmed gently to about 45°C The desiccator is connected to a suction pump and evacuated slowly until the water boils, and then the plates are placed in the desiccator

Just before boiling ceases, the desiccator is

sealed for the desired length of time The rate of

evacuation or more are required for completion

The method is, therefore, a combination of evacuation

the air which has, as compared with the usual method

that it is the same substance as that in which the

medium is placed, no expense or special equipments are required

the atmosphere saturated with water vapor

of the agar, no matter how long the period of

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building,
Richmond, Va.

Nutrition and Diet in Health and Disease^{*}

THIS is a book which the physician in general should welcome with enthusiasm for if there is anything concerned with the practical application of dietetic principles in health or disease which he will not find discussed its omission will be without significance.

With the help of this book the frequent inquiry, "what may the patient have to eat?" can be answered with specific detail and not in the general terms so often meaningless to the family.

The book is divided into three parts.

Part I (280 pages) discusses completely in a thoroughly understandable way the need for food and its utilization, food products, and diet in health, including an excellent discussion of the feeding of infants, children of school age and adults.

Part II (295 pages) takes up nutrition in disease from the standpoint of diseases in which diet is of paramount importance and diseases in which diet is of varying importance. In all instances the directions are detailed, clear and specific including numerous menus and recipes.

There is an excellent chapter in this section on diet in obesity and leanness which is well worth studying in the absence of indiscriminate enthusiasm for reducing.

Part III (87 pages) consists of tables and charts of a general nature.

There are few books on diet which should prove more useful than this to the practitioner or the dietitian. It can be highly recommended.

Clinical Atlas of Blood Diseases[†]

THIS volume, while small contains a large amount of material of great value to the physician.

The authors have attempted to produce the essential features of a textbook of hematology as well as an atlas showing the striking features of blood dyscrasias.

It is not intended of course, to be encyclopedic but rather to present in succinct form information applicable to the recognition and differentiation of the more common hematologic findings in disease.

The book begins with a glossary of the terms encountered throughout the book. Then follow five colored plates concerned with the normal and abnormal blood cells and their development. Nuclear indexes are then touched upon briefly, the remainder of the book being devoted to various diseases in which blood changes are encountered, marked or significant.

The colored plates are well done and excellently reproduced.

The book should be well received by the physician and student and will undoubtedly be used as a reference.

Nutrition and Diet in Health and Disease By J. S. McLester, M.D., Professor of Medicine, University of Alabama. Cloth 783 pages, numerous tables. W. B. Saunders Co., Philadelphia, Pa.

Clinical Atlas of Blood Diseases By A. Pliny Research, Pathologist, Cancer Hospital, London, and S. Wyard, Physician, Bellingbroke Hospital, London. Cloth 89 pages, 36 illustrations of which 32 are in color. P. Blakiston's Son & Co., Inc., Philadelphia, Pa.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

Determinative Bacteriology^{}*

THE Lehmann and Neumann Atlas of Bacteriology with its wealth of invaluable and unique colored plates needs no introduction to the laboratory worker

This new and extensively revised seventh edition, excellently translated by Dr Breed, will undoubtedly receive a warm welcome not only from those to whom the work is new, but also from those who have long used the previous editions

In this first volume, as before, are presented a large number of colored illustrations of bacteria as well as a brief but comprehensive section on technical methods

One can only hope that Volume II will not be long in following its predecessor

The Penicillia[†]

DESPITE the fact that the penicillia on the one hand are important agents for the ripening of cheese, and on the other, frequent sources of spoilage in fruits and various food stuffs, attack fibers, wood and paper, contaminate cultures in the bacteriologic laboratory, and at times are etiologic agents of disease in the human being, very little systematic or detailed information concerning their characteristics and activities is available

One cannot, at first, appreciate the incalculable labor entailed in the accumulation and classification of the information which has made this book possible. It represents the work of twenty years

The volume deserves and doubtless will receive a warm welcome from mycologists as well as all those in whose field penicillia are encountered, because of the tremendous amount of information it contains concerning this species. As far as is at present possible it furnishes for the genus penicillia information analogous to that found in determinative bacteriologic manuals

The Nursing of Infectious Diseases[‡]

A VERY well written small volume which physicians can safely recommend to nurses who are attending cases of contagious disease. After a general discussion of infection, the prevention of contagion, and the general management of infectious diseases, the following specific infections are discussed, scarlet fever, diphtheria, measles, German measles, smallpox, chickenpox, typhoid fever, whooping cough, meningitis, poliomyelitis, epidemic encephalitis, tuberculosis, and the venereal diseases

Those forms of treatment have been discussed which are usually left to the nurse to carry out, or for which she must make special preparations

Nerve Tracts of the Brain and Spinal Cord[§]

A COMBINED anatomy, physiology, and organic pathology of the tracts of the central nervous system. Profusely illustrated. This volume differs from the few outstanding works on central nervous system anatomy, in that it contains much on applied anatomy or neurology, and should be of distinct value as a reference manual in neurologic diagnosis. The illustrations, exemplifying attitudes, postures, and movements in the different organic neurologic diseases are especially useful

^{*}Determinative Bacteriology Vol I Atlas Prof Dr K B Lehmann and Prof Dr R O Neumann Ed 7 English translation by Dr R S Breed Cloth 65 pages of colored plates G H Stechert & Co (Alfred Hafner) New York

[†]The Penicillia By Charles Thom Mycologist Bureau of Chemistry and Soils Cloth 643 pages 99 figures Williams & Wilkins Baltimore Md

[‡]Lectures Upon the Nursing of Infectious Diseases By F I Woolcott M A M D B Ch (Oxon) D PH (Senior Assistant Medical Officer Grove Hospital Metropolitan Asylums Board) Revised and Enlarged by Dorothy C Hare CBE M D M R C P D PH Assistant Physician Royal Free Hospital Examiner in Medicine for State Examination Under the General Nursing Council Cloth pages 195 C P Putnam's Sons New York 1930

[§]Nerve Tracts of the Brain and Cord Anatomy Physiology Applied Neurology By William Keiller F P C S Professor of Anatomy and Applied Anatomy University of Texas Cloth pages 456 New York, The Macmillan Company 1927

The Autonomic Nervous System^{*}

THE advent of surgery in the domain of the autonomic nervous system has of recent years produced increased clinical interest. The volume under review is an authoritative compilation of what we know about the autonomic system today. Two thirds of it are taken up with anatomy and physiology and the remainder with its pathology and clinical study and treatment.

The term *involuntary nervous system* was first applied nearly two centuries ago but this term is inappropriate because many of the reactions of the so called voluntary nerves are not subject to voluntary control and because the nerves supplying certain of the visceral organs are not wholly free from voluntary influences.

The term *sympathetic* was introduced in 1732 by Winslow who believed that a sympathetic nerve system coordinated the functions of the different organs and that the sympathies of the body were brought about through these nerves. Another term *ganglionic nerves* suggested by Johnstone in 1764 has not lasted.

Fichat proposed the term *vegetative nervous system* in 1800 because he believed that the life of the organism was made up of two types of life: animal life and organic vegetative life.

Langley in 1896 proposed the term *autonomic* although realizing that this term suggested a much greater degree of independence of the central nervous system than in fact exists.

There is an excellent critical discussion of the results of sympathectomy and ganglionectomy operations as they are employed in various diseases today.

Outlines of Pathology†

WHILE this is a textbook on pathology it is in no sense the usual dry classification and morphologic and structural discussion. The author has endeavored with great success to make his pathology a living subject. Indeed he seems to have taken as his thesis the concept that the pathologist instead of dealing with death and with dead structures is dealing with life and living structures; that diseases are physical processes of the body and as much a part of life as health.

It is a rare experience to pick up a textbook of pathology and feel impelled to read on and on. This is such a work. We might well describe it as a pathology written by a philosopher. It should be most widely read.

Endocrine Disorders‡

A SMALL practical monograph on the clinical study and treatment of endocrine diseases. While it touches on disease of the various organs its greatest value to the clinician will be in its discussion of the growth dystrophies. The average man of intelligence has no difficulty in recognizing obesity but is often a bit at sea as to whether it is of pituitary or gonadal origin or due to other endocrine derangement. The volume under review will aid in classification of this type of condition and offers helpful therapeutic suggestions in so far as therapy in this field is of value.

^{*}The Autonomic Nervous System. By Albert Kuntz, Ph.D., M.D., Professor of Anatomy in St. Louis University School of Medicine. Illustrated with 70 Engravings. Cloth, pages 576. Lea & Febiger, Philadelphia, 1919.

†Outlines of Pathology in its Historical, Philosophical and Scientific Foundations. A Guide for Students and Practitioners of Medicine. By Horst Oertel, Strathcona Professor of Pathology, McGill University, Montreal, Canada. Cloth, pages 470. Montreal: Renouf Publishing Co., McGill College Avenue.

‡Endocrine Disorders. By Professor Hans Curschmann, Director of the Medical Clinic, University of Rostock, I.M. With an Introduction by Franz Prange, Doctor of Medicine and Philosophy, Assistant at the Medical Clinic, University of Rostock, I.M. Humphrey, Milford. Cloth, pages 188. Oxford University Press, American Branch, New York, 1929.

*Clinical Physiology**

UNDERGRADUATE physiology stands as a preparation for our understanding of clinical medicine. Too often, when we have completed the latter we have forgotten much of the details of the former. The volume by McDowall is offered we may say as an advanced physiology, presupposing an understanding of clinical medicine. It is in essence an applied physiology, the physiology of disease. It covers somewhat the same lines as that followed in Hewlett's Functional Pathology.

Diagnostic Methods and Interpretations in Internal Medicine†

THIS is, in essence, a work on physical diagnosis and the interpretation of laboratory findings. Considerable space is devoted to the technic of physical examination and special manipulations such as lumbar puncture and to the interpretation of observations. A small section is devoted to the form of the periodic health examination. The volume is quite complete and abundantly illustrated.

The Conquest of Cancer‡

THIS volume may be divided into two general subdivisions, first a discussion of the nature and cause of cancer, its pathology, the manner in which it affects different organs, its treatment by methods other than radium, and, second, the radium treatment of cancer. Some space is also devoted to the use of radium in nonmalignant diseases.

The author believes that surgery is of benefit in about 50 per cent of cancer cases, x-ray in 10 per cent, and radium either alone or in conjunction with surgery or x-ray in a very much higher proportion. In advanced cases where it is a matter of relieving pain, lengthening life and minimizing the disagreeable symptoms, x-ray assumes an importance equal to surgery and radium.

He recommends surgery in cancer of the alimentary tract, except the rectum, mouth, throat, and esophagus, cancer of the central nervous system, bones, the breast, the body of the uterus. In cancer of these organs, however, he believes that operation should be preceded by the use of x-ray or radium.

X-ray is recommended in conjunction with radium in rapidly growing sarcoma and in conjunction with surgery and radium in cancer of the breast.

Radium alone is preferred in all cancers of external parts, the cervix, the bladder, and malignancy of the mouth, throat, and esophagus and rectum and in sarcomas and uterine fibroids. The latter group comprises about 40 per cent of all neoplastic growths.

*Clinical Physiology (A Symptom Analysis) In Relation to Modern Diagnosis and Treatment. A Text for Practitioners and Senior Students of Medicine. By Robert John Stewart McDowall D.Sc. M.D. F.R.C.P. (Edin.) Professor of Physiology, King's College, University of London. With an Introduction by W. D. Halliburton LL.D. F.R.C.P. F.R.S. Emeritus Professor of Physiology, King's College, University of London. Cloth, pages 383. New York: D. Appleton and Company, 1927.

†Diagnostic Methods and Interpretations in Internal Medicine. By Samuel A. Loewenberg M.D. F.A.C.P. Assistant Professor of Clinical Medicine, Jefferson Medical College, Assistant Physician to the Jefferson Hospital. Visiting Physician to the Philadelphia General Hospital, The Northern Liberties Hospital and the Eagleville Sanatorium for Consumptives. Formerly Assistant Professor of Physical Diagnosis at the Medico-Chirurgical College and the University of Pennsylvania, Philadelphia. With 547 illustrations, some in colors. Cloth, pages 1032. Philadelphia: F. A. Davis Company, Publishers, 1929.

‡The Conquest of Cancer by Radium and Other Methods. By Daniel Thomas Quigley M.D. F.A.C.S. Instructor in Surgery in the University of Nebraska College of Medicine, Fellow of the American Medical Association, Member of the American Association for the Advancement of Science, Nebraska Academy of Science, North American Radiological Society, American Radium Society, Fellow of the American College of Radiology, etc. Director of the Radium Hospital of Omaha. Illustrated with 334 engravings. Pages 639, cloth. Philadelphia: F. A. Davis Company, Publishers, 1929.

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EDITORIALS

The Sugar Content of the Blood

FOR more than fifteen years we have possessed methods for the estimation of blood sugar which have been adequate for practically all clinical purposes. At present the choice of satisfactory methods available to the clinic is very large. Although these methods depend upon a variety of analytical principles, colorimetric, volumetric, gravimetric, gasometric and polariscopic methods having been used, most of them have been built around the reducing action of glucose, in particular upon copper reagents. It is of interest in this connection to note that as far back as the time of Claude Bernard a violent discussion arose as to the reliability of copper reduction tests in the estimation of the sugar of the blood.¹

With the introduction of our present blood sugar methods, most of which are sufficiently simple to permit their use in the clinic, it was generally conceded that the results obtained were probably slightly too high due to the interference of other reducing substances in the blood. It was quite justly felt that it mattered little whether the normal blood sugar was actually 100

mg per 100 c c or only 80 mg, as long as the method gave consistent results. It was argued that the greater part of the reduction must be dependent upon glucose, and this was unquestionably the chief variable. Furthermore in diabetes, the clinical condition in which the determination was most used, a rise of 50 to 200 per cent was generally encountered and might reach 1000 per cent. Obviously the reducing nonsugars could not have an appreciable influence on such changes.

Slight hyperglycemias may be observed in other conditions notably in hyperthyroidism and nephritis. Some workers have questioned the reliability of the somewhat higher findings in nephritis ascribing the increased reduction to the influence of the retained nitrogenous waste products. While some of the bodies, notably creatinine may influence the results obtained, they do not by any means account for the hyperglycemia observed, and furthermore, quite marked fluctuations in blood sugar may take place with little change in the known nitrogenous constituents.² Recently Lyttle and Hearn³ have compared the Folin-Wu and Benedict (copper I) methods on bloods showing nitrogen retention and found that the nonprotein nitrogen of the blood bore no relation to differences in the sugar content observed by the two methods. It may further be noted that the carbohydrate tolerance is decreased in both nephritis and hyperthyroidism, and the blood-sugar tolerance curves obtained resemble those found in mild diabetes.

It has long been recognized that in hypoendocrine conditions, the pancreas excepted, comparatively low blood sugars are encountered. Hypoglycemias have been observed in myxedema, cretinism, Addison's disease, pituitary disease and also in some diseases of the liver, such as acute yellow atrophy and certain forms of cirrhosis. Very low blood-sugar values may be encountered after overdoses of insulin. Do the convulsions and other symptoms occur when there is an appreciable amount of glucose in the blood, or only when the supply of glucose is completely exhausted? It is obvious that such questions can only be answered when data are available for the true glucose content of the blood.

The name of S. R. Benedict has been linked with every great advance in our sugar methods during the past twenty years. In 1909 he introduced his single qualitative reagent now almost universally employed as a qualitative reagent for sugar in urine and this was followed two years later by his quantitative reagent. In collaboration with Lewis⁴ he introduced our first colorimetric method of blood-sugar estimation in 1913, final publication of the method appearing in 1915. Some years later, realizing that the various blood-sugar reagents were apparently reduced by substances other than glucose, he⁵ attempted to prepare a copper reagent which would be more specific for glucose and in 1925 described a reagent which when applied to the Folin-Wu tungstic acid filtrate, gave results definitely lower than those obtained by the Folin-Wu method.

Folin⁶ admitted that the lower results obtained by Benedict were probably nearer the true values for glucose, but objected to some of the chemical principles underlying Benedict's new method. He suggested a reagent which he thought preferable but one that gave essentially the same results. At about

the same time Harned⁷ applied the Folin Wu method to blood which had been precipitated by a mercuric reagent, which removes practically all the nitrogen and obtained values in essential agreement with Benedict's new copper method

Almost simultaneously with the publication of Benedict's copper Method I, Van Slyke and his coworkers⁸ made an important contribution by showing that the residue of reducing substances after yeast fermentation amounted to 10 to 30 mg per cent in terms of glucose. The use of yeast in the determination of the nonsugar reducing substances in blood has been employed to advantage by Folin and Svedberg, Somogyi, and Benedict. Folin and Svedberg⁹ found that with the new Folin method and nonfermentable reducing substance amounted to 5 or 6 mg per 100 cc whereas with the Folin Wu method it was larger (about 22 mg judging from their data). Somogyi has published several papers dealing with the nonsugar reducing substances of the blood, following yeast fermentation. With Kramer,¹⁰ he found that the reducing nonsugar, as determined by the Shaffer Hartmann method, amounted to about 22 mg and that the determination of the true sugar by three different methods yielded essentially identical values. Somogyi¹¹ further observed that more than 75 per cent of the nonsugar reducing substances was in the cells, the average values on 36 bloods being 8 mg for the serum and 40 mg for the corpuscles.

Apparently feeling that his copper Reagent I fell just short of measuring the glucose content of the blood, Benedict¹² elaborated another copper Reagent II which he believes gives very closely the true glucose content of the blood when applied to the Folin Wu tungstic acid blood filtrate. As a result of fermentation experiments and observations with this new method, Benedict concludes that the Folin Wu technique yields figures for the blood sugar which average about 22 mg per 100 cc of blood too high.

It is a well known fact that the Hagedorn Jensen¹³ method so much used in Europe, gives somewhat lower results than most of the methods employed in this country. This method employs a zinc salt in the precipitation of the protein. Somogyi¹⁴ has carried out blood sugar determinations on the blood filtrates after a similar zinc precipitation and obtained essentially the same results with four different methods on 35 separate blood samples indicating that the zinc precipitation removes the nonsugar reducing substances. The methods used were the Shaffer Hartmann (modified), Folin Wu, Folin, and Benedict II.

From the foregoing it is apparent that the older blood sugar methods such as the Folin Wu and Shaffer Hartmann give results which are approximately 22 mg too high while the Lewis Benedict (and the Myers Bailey, and Benedict modifications) yield figures which are probably slightly higher.

Now that it appears to be definitely established that normal blood contains about 22 mg of nonsugar reducing substance per 100 cc of blood one naturally raises the question as to the nature of this reducing substance or substances. From the work of Somogyi it would appear that more than 75 per cent of the reducing nonsugar is present in the corpuscles. In the same issue of the *Journal of Biological Chemistry*, Benedict and Newton¹⁵ have re-

ported observations in harmony with this finding, which go a long way toward explaining the 22 mg of nonsugar reducing substance obtained with the Folin-Wu method. They are of the opinion that the 50 to 100 mg of glutathione presumably present in 100 c c of blood exercise a large part of this reducing power and have presented some data in substantiation of this. They do not believe that the thionine present ordinarily accounts for more than 1 or 2 mg of reduction per 100 c c of blood, although occasionally it might amount to 4 to 5 mg.

It would appear therefore that the very perplexing problem as to what was the true sugar (glucose) content of the blood, which has been a topic of discussion from the time of Claude Bernard, had practically reached solution, and further that we will not have long to wait for a satisfactory explanation of the nonsugar reducing action of blood.

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—V C M

The Significance of Achlorhydria in Pernicious Anemia

OFTEN the discovery of the cause of a disease leads to improvement in treatment. New methods of treatment of syphilis followed rapidly after the demonstration of the specific organism of the disease. Occasionally this sequence of events is reversed and the successful treatment of a disease results in new light on etiology as illustrated by new developments in pernicious anemia.

The exact cause of pernicious anemia is not settled. Recent work offers, however, much evidence relative to the etiologic factor or factors concerned in the characteristic clinical symptoms and pathologic picture. The two constant findings in the disease, which together are almost pathognomonic, are the achlorhydria and the macrocytosis of the red blood cells. The macrocytosis is an expression of a difficulty in maturation or normal growth of the erythrocytes. The exact meaning of the achlorhydria has not been clear although every student of the disease considers the absence of free hydrochloric acid in the stomach as a clinical finding necessary for the diagnosis of the disease. When pernicious anemia was considered a hemolytic disease, it

seemed probable that the development of a toxin responsible for the blood destruction might be facilitated by the absence of free acid in gastric digestion. Certainly the hemolytic conception of the disease and probably all toxin theories of the disease must be given up. The primary trouble in blood regeneration is probably the lack of some substance which normally influences the bone marrow and is necessary for normal red cell formation.

The demonstration by Minot and Murphy¹ of a specific antianemic factor in liver has not solved the problem of the actual cause of the disease, although offering convincing evidence that the disease is a deficiency one. It is recognized that the principle is not specific to the liver although this organ is still the most satisfactory source of supply. Many other organs contain it. Recently it has been shown by Zerfas and Koehler that adrenal extract may have a stimulating effect similar to liver extract. The primary source of the specific principle is as yet unknown. It is quite possible for instance that the liver is only a storage point and not a site of formation. New developments throw some light on this phase of the question.

Castle, in studying the relation of achlorhydria to pernicious anemia, found that the stomach of normal persons secreted a substance which could develop from meat the principle necessary for the maturation of red blood cells. Meat when digested with normal gastric juice gave the typical reticulocyte response when fed to patients with pernicious anemia. Beef muscle digested with hydrochloric acid and commercial pepsin gives no such an effect. These results suggest in view of the constancy of the achlorhydria that the principle responsible for the normal maturation of red cells is a gastric secretion other than hydrochloric acid or pepsin. The achlorhydria is only an evidence of this lack of some principle responsible for the disease rather than being itself responsible for the disease.

Later work adds further evidence of the truth of this view. Sturgis and Isaacs³ and Sharp⁴ have found that desiccated stomach of the hog when fed to patients with pernicious anemia produces the blood changes characteristic of liver extract and a remission in the disease. Conner⁵ reports similar results. Sturgis and Isaacs conclude that "the observations so far are in accord with the idea that patients with pernicious anemia evidently have lost or have never had the ability to secrete a substance in their stomachs which has the power to produce blood maturing substance from food." Thus the evidence accumulates that pernicious anemia is a deficiency disease. The lack of a necessary principle leads to difficulty in bone marrow function as evidenced by the macrocytosis of the red cells. The evidence further indicates that the deficiency is primarily a gastric one, the disturbance in gastric secretion being shown objectively by the achlorhydria.

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SYMPOSIUM ON ARTHRITIS

THE PRESENT STATUS OF THE PROBLEM OF ARTHRITIS

BY RALPH PEMBERTON • MS MD PHILADELPHIA

THE problem of arthritis has recently entered upon a new phase of development. Stagnant and neglected for many years interest in it has been revived and has become active. Clinics for the study and treatment of the disease have arisen within the last three or four years in all parts of the country.

It may be well to enumerate briefly at the outset the chief developments which have taken place in regard to the problem of arthritis both internally and externally. They are as follows:

Recognition of the economic importance of arthritis with consequent pressure from the industrial world that more be done about it, establishment of the American Committee for the Control of Rheumatism for cooperation in an almost world wide investigation of the question, general recognition of two main morphologic types of the disease, recognition of hereditary and constitutional influences together with the existence of prodromata which often allow the disease to be anticipated, evaluation of the problem of focal infection to which extreme values have often been attached, renewed investigations of joint tissues for infecting organisms, appreciation of the existence of a disturbance of physiology, the so called dynamic pathology which is at least partly responsible for the actual morphologic changes and symptomatology of the disease, recognition of the rationale back of certain useful forms of therapy, extension of the application of these forms of therapy and the development of new measures along the same lines, fuller evidence leading to appreciation of some of the factors operative to produce and perpetuate the disease especially through the intestinal tract together with various therapeutic implications attaching to this, wider recognition of the influence of body posture upon develop-

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istence of many conditions and perhaps in no other more than in arthritis. Even when it does so act, however, the role is one of precipitation rather than, fundamentally, of causation. The world is full of individuals surcharged with focal infections yet wholly free from arthritis. Nor does removal of infections necessarily make the arthritic well or even better. Medical interest has been focusing too long upon the caliber of the bullet which killed the man in the holdup, and too little upon the state of society which encourages and permits holdups to occur at all. It can be made clear today to any dispassionate mind that factors apparently unrelated to bacteriologic activity and infection, in the usual sense of the word, may bring about the symptoms of arthritis of either atrophic or hypertrophic type.

Following recognition of the importance of focal infection Rosenow, Haden, and others, many years ago, isolated various organisms, chiefly streptococci, from the joints and other tissues. Recently Shands et al., have recovered streptococci, staphylococci and even the gonococcus from the joints and neighboring tissues. Cecil has described a strain of streptococcus which he regards as causative and Small entertains comparable views toward the indifferent streptococcus, isolated by him from cases of rheumatic fever and renamed by him according to Hitchcock 'the streptococcus cardioarthritidis.

There can be little doubt that, precisely as arthritics may harbor many foci of infection so they may sometimes harbor any of several organisms in the joints or other structures. Actual purulent joints are met with, although very rarely in chronic arthritis. Whether these organisms, however, are there in a causative relationship or more in the role of invaders from without is not so clear. Certain it is that apparently normal people may harbor massive focal infection. The factor which makes focal infection operative to produce arthritis is farther to seek and it is therefore equally clear that consideration must be given to the factors which permit or induce invasion of joint or other tissues by bacteria in arthritis when indeed this does actually occur.

The many uncertainties in this vexed field, the division of views, the contradictory results of bacteriologic study and especially the case of contamination have given rise to the increasingly accepted viewpoint that the bacteriologic influence in arthritis may be referable partly or largely to an allergic basis.

Space forbids development of this complicated problem and some consideration must be given to the changes in the fluid and fixed tissues which accompany, and are at least partly responsible for, the clinical and morphologic phenomena of the disease.

A long series of studies has been carried out by my associates and myself² which helps to explain the nature of the disease and forms the basis for certain advanced types of therapy. Without going into details it may be said by way of summary that the subject of chronic arthritis, of either type tends to present, in the absence of fever, a slight lowering of basal metabolism. This is not due to any inborn error of metabolism *per se*, but apparently to curtailment of the circulation to those tissues especially the muscles which have to do with processes of oxidation. Another result of this curtailment of blood supply is a delay in the rate of removal of substances circulating in the blood stream. Glucose is an example of such a substance and, for the reason given brings

about when fed to patients with arthritis, in large amounts, a "delayed rate of removal", erroneously called in this connection a "lowered sugar tolerance". Other observations by Peirce and myself which cannot be described in detail, reveal that the peripheral blood count of the patient with arthritis shows a diminution in the number of red cells in the blood first issuing from a stab, because of the constricted or at least empty condition of the capillary bed. This is further borne out by observations with the thermocouple which show beyond any doubt that the patient with arthritis usually maintains at the periphery a lower temperature than does a normal person. The amplitude of variation of his peripheral temperature is furthermore less than is that of the normal man. This is brought out by subjecting the patient with arthritis to conditions of cold, under which his surface temperature falls less and, afterward, returns more slowly toward normal. This relative immobility of the vascular bed, where the cause of this phenomenon is apparently to be found, explains much of the added disability experienced by the patient with arthritis from those changes in his environmental circumstances occasioned by the weather.

Again, direct observation of the capillary bed of the patient with arthritis under the microscope shows that the vessels present an intermittent and often sluggish blood flow and that they are often relatively empty, sometimes nearly invisible and of a shape differing from the normal. Direct observation of this field under the influence of measures which benefit the arthritis, such as massage, heat, exercise, aspirin, and the like may bring about a change in the picture of a highly graphic nature.

In view of these more recent findings it can be mentioned that careful studies of the blood gases in arthritis will show that in a certain proportion of cases there is a slightly increased percentage saturation of oxygen which is apparently due to the fact, referred to above, that the circulating blood inadequately reaches some of the tissues normally concerned in removing certain constituents from it. An interesting corollary to these observations is the fact that by cutting off the blood supply to the patellae of dogs, it has been possible to produce definite overgrowth of the patella,² resembling closely hypertrophic arthritis corroborating earlier experiments along the same line by Wollenberg.

The general point of view here developed has been progressively entertained for about eight years by the writer and his associates.⁴ Recently some corroboration of its validity has been forthcoming from another quarter, namely, the influence of the operation of sympathectomy upon certain selected arthritides of advanced type. Adson and Rowntree have shown unmistakably that following this procedure the return of blood supply to the involved parts may be accompanied by striking evidence of subjective and objective benefit.

There is in these several considerations obvious explanation of the rationale underlying the influence of many measures long known to be of value in arthritis. Thus I and my associates have for some years been studying the influence of heat and massage upon the arthritic syndrome. Omitting details, it seems clear that this highly beneficial influence is referable to the increase and betterment of circulation not only in the parts chiefly concerned but also in a more systemic sense.

There must now be mentioned a line of observations which bears almost equally upon the etiology, pathology, and treatment of arthritis. Many years ago I became aware of and described the unusual conditions of the intestinal tract which are present in the course of chronic arthritis and often precede it. This condition is especially to be seen in the colon because it can there be most satisfactorily studied by the x-ray and consists in elongation, widening, tortuosity, and inertia of that organ.

These observations were secondary to the attempt to elucidate the influence of a reduced food intake, first emphasized by me in 1912.⁵ This subject has recently had interesting and stimulating emphasis at the hands of Fletcher who has shown that, pursuant to proper lines of therapy, the bowel may return to ward or to a normal condition coincidentally with clinical betterment of the arthritic patient as a whole. The means to this end have been dietary and Fletcher lays emphasis upon three main facts: curtailment of the carbohydrate intake, a large vitamin content and adequate ingestion of protein. It is of great interest to note in this connection that McCarrison,⁶ Rowlands,⁷ and others have produced in animals precisely the intestinal picture encountered in arthritis, by means of avitamin diets coupled with a large intake of carbohydrate. If the carbohydrate be reduced or if the vitamin be adequate, the pathologic picture does not result nor does it do so if there be an adequate supply of protein. It has been clearly shown that on this basis infection implants itself readily in many tissues.

The wealth of philosophical considerations contained in these several experiments needs no emphasis. It is plain that factors of a nature much more generic than most of the profession have realized are operative in the production of the rheumatoid syndrome. Furthermore it shows that therapy along orthodox lines, regarded until recently as adequate, may be wholly wide of the mark. This undoubtedly accounts for the failure which has resulted on such a widespread scale following what appeared to be the proper removal of apparently causative focal infection.

As occasionally happens when the way has been pointed out, the laity, sooner than some of the profession, themselves find out where relief can be obtained. They have therefore begun to flock in large numbers to those obliging persons, largely technicians, often enthusiasts who will give them the treatments they desire. In this particular instance it becomes partly a question of betterment of function of the intestinal tract through colonic irrigation. Although this measure, under proper precautions, has benefit for many persons it constitutes only a part of the story. That which is removed by irrigation is of course chiefly that which is introduced by mouth. On this point, in part, and upon others having to do with the metabolism under conditions of an under-maintenance diet, hangs the propriety of dietetic therapy. The question is too large to be entered upon here except to emphasize its importance and indicate where fuller references can be found (loc cit). Such therapy involves elements of demerit as well as merit unless there be familiarity with the principles of nutrition but the results in properly chosen cases are usually striking.

Another chapter to which scant attention has been given by the as a whole is that relating to the extent to which congenital or ..

posture paves the way for, or actually brings about, chronic arthritis. Through visceroptosis and the many causes leading up to it, the intestinal tract achieves, in these improperly made persons, the condition referred to above. Adequate appreciation of this influence makes it often possible to prevent the occurrence of arthritis in persons at large, best illustrated in certain members of families in which the affliction of some members has clearly indicated the probable involvement of others. Furthermore, the sequelae of arthritis may be as serious as the disease itself and when they exist they may constitute additional causes of the disease. The splendid lesson taught by the Boston Orthopedists points the way to break this vicious cycle and to institute in its place a constructive program whose results will delight the heart of every honest and conscientious physician.

There is no opportunity here to enter upon the question of treatment as a whole. It must appear, however, from the above considerations that no single measure suffices to cover this extensive field. For the individual who is so constituted as to make arthritis a possible and early episode in his life, infection may be only as the drop of the flag which starts the race, the stage for the spectacle being set. The inevitable corollary to this is, that while such measures as vaccines have their legitimate place in medicine and also in the treatment of arthritis, they can at best, influence only one factor, if indeed, in arthritis, they often do that. Granting them in arthritis even greater success than they are accredited by sound clinicians, they can in no way change the background on which the disease starts or the consequence of its inroads, and, as a rule, they do not alter the disturbance of physiology which brings about the lesions themselves. I am desirous of making it clear that it is not my purpose to extol or depreciate any one of the many sound measures of therapy available, whether removal of infection, physiotherapy, use of drugs or diet, improvement of intestinal function, betterment of body posture, or what not. Each of these has its place in the complicated program necessary in any attack upon the arthritic problem. To consider arthritis, however, as a disease for which any drug, vaccine, operative, or other procedure is the remedy is to fail to see the problem whole. Now that the medical outlook upon this disease is widening and many new data of precise and clinical nature are available, there is no excuse for approaching this syndrome along the lines of single-minded enthusiasm or prejudice. The time is not far distant when treatment of the hosts of patients with arthritis in the United States, under any outlook which does not comprehend the many important factors in the problem, will be tantamount to a form of malpractice. Indeed, it is so now in the minds of dispassionate students of the subject. An illuminating corollary of this is to be seen in the fact that the various persons who from time to time advocate drug A or vaccine B as affording a specific attack on arthritis, gradually, of necessity, extend their armamentarium when their proclamations have achieved a substantial practice. With the increasing failures which adherence to a limited slogan inevitably brings about, they are driven to utilization of all the measures at the hands of the profession. Eventually these persons usually become saner and more rounded out, and, like the homeopaths who have widened their curriculum

to where it approximates that of the regular school, excuse for and practice of their specific tenets ceases

One therapeutic consequence of large importance follows upon recognition of the two chief types of the disease, namely that atrophic arthritis tends to ward ankylosis and that hypertrophic arthritis does not. Some rest is usually indicated in both varieties but the extent to which it is applied and the accuracy with which the arthritis itself is classified, may determine whether an ankylosed or movable joint is to result. While certain forms of treatment cluster around each variety of arthritis however, there is no proved justification clinical or laboratory, for denying patients in either group the benefit from measures of generic value because of a priori convictions of etiology.

The subject of arthritis is curiously enough by way of becoming so popularized that whereas it was formerly difficult to get adequate attention from the profession for these sufferers now persons and clinics are turning to this disease in such numbers with not infrequent immaturity of viewpoint, that it becomes necessary to introduce a restraining hand and advocate dispassion. Arthritis touches more fields of medicine than does any other disease with the possible exception of syphilis. For this reason its manifold subtleties cannot be learned easily or quickly. In consequence, snap judgments are all too common and the long-suffering laity will continue to suffer until single-minded enthusiasts have shot their bolts and the main body of the profession has caught up. No one is more conscious than I am of the great desirability of more information in this vexed field. By the same token, however, no one is more conscious of the large amount of precise evidence of laboratory and clinical nature bearing on the disease, which already exists. This information is available to anyone who has the interest and pertinacity to seek it. There is perhaps no more important problem before the medical profession today, in terms of persons now living than that of the desirability of extending this information to the great army of arthritics. It is little short of tragic to realize that countless sufferers from arthritis must approach the grave in wheel chairs merely because available information is not yet sufficiently widely diffused. Under proper conditions of therapy the statistical probabilities of benefit to any given patient with arthritis are definitely in his favor. There are in fact few other chronic diseases for which more can be done.

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RECENT STUDIES OF RHEUMATISM, WITH SPECIAL REFERENCE TO GONORRHEAL ARTHRITIS*

BY RALPH A. KINSELLA, M.D., ST. LOUIS, MO

ONE of the interesting changes in medical teaching during the past decade has grown out of the tendency to unify ideas concerning pathologic states

In previous years students of medicine learned about peripheral diseases and disregarded the common ground that lay between these varied disturbances they learned about nephritis and arthritis, and myocarditis, and gastritis, and disregarded the fact that between many of the diseases which they studied separately, there was a structural connection which itself was primarily responsible for the diseases in the separate peripheral organs

One of the expressions that recognized this idea early was "cardiovascular-renal disease"

A still later tendency has been to emphasize the vascular tree as the primarily diseased structure and to study the conditions which bring about acute and chronic inflammatory and degenerative processes in the arterial system

This tendency weakens the idea of specificity to some extent and makes it unnecessary to believe that a certain selective activity is required for the joints to be involved, a certain selective activity for the myocardium, a certain selectivity for the kidneys, and so on. On the other hand it emphasizes the necessity for studying the changes in blood vessels more closely to discover the differences in structural disturbances between a disease like acute rheumatic fever and a disease like hypertensive vascular disease, which has no articular symptoms

Acute rheumatic fever is one of the diseases in which the involvement of blood vessels has been found throughout the body, affecting organs which yield no symptoms as well as parts that produce the greatest clinical display

The studies of Swift and of Von Glahn have identified the perivascular lesions in the neighborhood of the joints with those in the heart muscle, the subcutaneous nodule, and the kidney, in this disease. Thus, a disease which students previously regarded as a selective involvement of joints, is found to be not at all a disease of joints but a disease of the vascular tree. So many lesions occur near the joints that the clinical signs of redness, heat, and swelling naturally follow

The experimental production of arthritis in animals is interesting in illustrating the extra-articular character of the disease and the fact that the disease is also one of blood vessels both in its early and late stages

In this study, hemolytic streptococci were injected into the ear vein of rabbits. At twenty-four-hour intervals after the injection the animals were

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killed and the tissues of the joints were examined microscopically to discover the place where the lesion about the joint was beginning and how it was developing

The results showed that the lesion began as a minute hemorrhage definitely outside the joint capsule and that it progressed as it changed into a purulent focus until several days later the joint cavity was penetrated and true arthritis established. The second result was that in its later stages the infection showed proliferative vascular changes in all the organs

Thus one form of bacterial disease affecting joints in rabbits was, in its early stages, an exudative vascular disease and in its late stages a proliferative vascular disease

Not all forms of bacteria will produce immediate results in the vicinity of joints following intravenous inoculation as will the hemolytic streptococcus and it is likely that in humans a period of time must intervene between the inoculation of bacteria and the production of rheumatism

During this period of pause, certain changes are taking place in the tissues of the host which have been referred to as allergy

To the researches of Swift we must look for the arguments which indicate that acute rheumatic fever is a disease which depends for its clinical manifestations on the establishment in the body of a state of allergy. The explanation which Swift gives includes the comparison of acute rheumatic fever with the allergic phenomena of tuberculosis

This mechanism of allergy is said to be produced in patients by means of an infection by any kind of streptococcus, such as tonsillitis, sinusitis or other local infections

While the tissues are still in this state of allergy, the further contact of the body with any streptococcus (or its toxin) will precipitate a critical reaction in which the small blood vessels participate, and a typical proliferative lesion (Aschoff body) results. The clinical expression of this reaction is the disease, acute rheumatic fever

Acute rheumatic fever is therefore not a disease produced by the initial inoculation of streptococci but, according to the Swift hypothesis is the reaction of an allergized body to further contact with a streptococcus. In other words, if the process of allergy, or the second contact, were avoided the disease might not be produced

Now in rabbits sodium salicylate will inhibit the development of allergy as expressed by the cutaneous reaction. The clinical usefulness of sodium salicylate in the treatment of acute rheumatic fever may depend on its capacity to suppress temporarily the process of allergy. Perhaps x ray would act in this way, and the beneficial effect of x ray on rheumatic carditis, reported by Levy, may be thus explained. Neither of these agents, sodium salicylate nor x ray can be regarded as antistreptococcal in its action and both are only partially helpful to patients

The use of vaccines and serums in the treatment of this disease is not yet satisfactorily developed

This interesting influence of allergy is no doubt at work in many infectious diseases which, until now, have been regarded as simply the reaction

of bodies inoculated with bacteria. Even a disease like lobar pneumonia is probably not produced suddenly in robust subjects, as textbooks frequently state, but more often the explosion follows a week or two during which there has been an alleigizing respiratory infection.

It is necessary, therefore, to bear in mind that while the injection of streptococci may be followed immediately by arthritic disease in rabbits, in human beings such an immediate response is by no means easy to observe.

Some intercurrent event is usually necessary to permit the invasion of the blood stream by bacteria which for weeks or years have been retained in a middle ear or a posterior urethra.

Hanger, Swift, and ourselves have shown that animals allergic to one type of bacteria may be killed by the intravenous inoculation of an entirely different and otherwise nonlethal kind of bacteria.

The clinical application of this idea is easily observed in cases of gonorrheal rheumatism. In the first place, the implantation and growth of gonococci in the urethra may or may not lead to a bacteremia but rheumatism is almost never the immediate accompaniment of urethritis. Nevertheless, the body becomes allergic to gonococci and so remains. The factor responsible for the later production of bacteremia and rheumatism is an intercurrent infection or intoxication.

During the past few months we have seen an upper respiratory infection exert this influence in three instances, a hemolytic streptococcus infection of the throat once, and food poisoning once.

Gonorrheal rheumatism is a bacterial type of rheumatism. While employing the mechanism of allergy for its production, this form of rheumatism depends for the continuation of its clinical manifestations on the presence of gonococci. It differs from acute rheumatic fever in its persistence and from all other pyogenic forms of rheumatism in its tendency to localize in one or two joints. It occurs in two chief clinical forms: a simple effusion into the joint, or a dense, tender induration of the extra-articular tissues. The place near the joint where the inflammation starts can be detected by the pink area in the skin which overlies the purulent focus. In the effusion types, fluid is easily obtained by puncture. Such fluid contains few gonococci. In the brawny indurations of the other type, fluid is difficult to obtain but in the drop of bloody pus on the needle-tip, gonococci are more easily demonstrated.

In the cases where a large effusion causes the joint to be distended, and where there is no evidence of extra-articular infiltration, the body is usually able to rid itself of the disease and the treatment consists only of tapping and extension of the joint. However, the body is not able to eradicate those extra-articular infiltrations which are rich in gonococci, and while it may receive help from various nonspecific forms of injections, the results are unsatisfactory. The worst treatment is the immobilization of such joints in casts or splints.

The best results are obtained in this group of cases by making an incision through the most congested and tender area to the site of the pus. Evacuation and drainage of this purulent focus has resulted in 20 of our cases, in complete recovery.

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BEAUMONT BUILDING

THE DIFFERENTIAL DIAGNOSIS OF RHEUMATOID AND OSTEARTHTRITIS THE SEDIMENTATION REACTION AND ITS VALUE*

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I THE DIFFERENTIAL DIAGNOSIS OF RHEUMATOID AND OSTEARTHTRITIS

THE study of chronic multiple arthritis has demonstrated the existence of two separate and distinct disease processes which are conveniently grouped under this general title. The first of these represents a multiple arthritis with additional evidence of a generalized tissue response to an infectious process, the second, a degenerative process involving the joint structures and appearing at a time of life when other degenerative changes are prone to occur. The existence of these two clinical entities is clearly recognized, but the obscurity surrounding their etiology has precluded the adoption of an adequate and generally accepted terminology. The following descriptive terms, employed in the literature devoted to this subject, still meet with greater or less favor

Synonyms for Group I	{	Rheumatoid arthritis
		Chronic infectious arthritis
		Atrophic arthritis
		Arthritis deformans (American usage)
		Rheumatic arthritis
		Chronic rheumatic arthritis
		Chronic rheumatism
		Primary progressive polyarthritis
	}	Secondary progressive polyarthritis

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Synonyms for Group II	{	Osteoarthritis
		Degenerative arthritis
		Hypertrophic arthritis
		Climacteric or menopausal arthritis
		Senile arthritis
		Arthritis deformans (German usage)

The title chosen to designate each particular variety is of small importance so long as it is recognized that the two groups represent distinct clinical and pathologic entities. For the purposes of discussion, however, it is essential that one particular terminology should be at least tentatively adopted.

The terminology most generally employed in America is either one of the following

- I Chronic infectious arthritis
- II Degenerative arthritis, or
- I Atrophic arthritis
- II Hypertrophic arthritis

The classification adopted by the British Ministry of Health is as follows

- I Rheumatoid arthritis
- II Osteoarthritis

The first of the American terminologies, that employed by Cecil,² possesses certain definite advantages. It emphasizes the distinction between the infectious and noninfectious forms of arthritis, a differentiation which we feel to be thoroughly justified. Moreover the term *degenerative* suitably describes the type of pathologic process involved in that particular form of the disease.

The British terminology also possesses distinct advantages. The term *rheumatoid* indicates that a relationship exists between this form of chronic arthritis and rheumatic fever, a relationship which we believe to be of great importance. The term *osteo*, while less apt, indicates that this form of arthritis is essentially a disease of the osteoid structures of the joints. In addition, the British classification possesses the advantage of the weight of great authority. Garrod,¹ in 1908, in his classical description of these diseases, adopted the terms *rheumatoid* and *osteoarthritis*. Since Garrod's time singularly little has been added to our knowledge of these conditions and confusion rather than clearness has resulted from the introduction of new terminologies. In the Arthritic Clinic of the Presbyterian Hospital we have elected to follow the British classification. This classification has been recommended by the International League for the Control of Rheumatism and therefore possesses the advantage of widespread use. The present communication is concerned with the differential diagnosis of these two groups of chronic arthritis.

A third and much less frequent type of chronic arthritis, variously designated as metabolic arthritis, gouty arthritis, or chronic gout is now recognized as a separate disease entity. The differential diagnosis of this form of chronic arthritis only causes difficulty in rare instances. With the aid of the following criteria, as pointed out by Hench,³ Cecil,² and others, the diagnosis can usually be made without danger of error.

- 1 Classical history of onset with an attack of acute pain in the great toe
- 2 The paroxysmal and intermittent nature of the acute and subacute attacks frequently associated with complete joint remissions
- 3 The occurrence of gouty tophi in the ears or about the joints
- 4 Increased uric acid content of the blood
- 5 Characteristic roentgenologic findings of punched out areas in the epiphyses of the bones. Such punched out areas however are not infrequently seen in cases of rheumatoid arthritis and this finding should only be considered of value when the areas are quite large and when associated with other manifestations of the disease

Another sign has frequently been described as characteristic of gout—namely the occurrence of nodular swellings in the subcutaneous tissue near the joint structures and along the course of tendon sheaths. Great care, however must be exercised in differentiating such nodules from similar structures which occur in rheumatoid arthritis.

The two major groups of patients represent entirely different clinical and pathologic states and the first step toward a more intelligent appreciation of

TABLE I

	RHEUMATOID ARTHRITIS	OSTEARTHRTIS
1 Family History	Not infrequently a history of rheumatic fever in an immediate member of family	Frequently a history of a similar form of arthritis in older members of family
2 Past History	Occasionally a history of rheumatic fever frequently a history of tonsillitis or sinusitis	Not characteristic
3 Age at Onset	Any age over 80 per cent between twenty and fifty	Rare before 40 most frequent 40-50 In women most common at menopause (menopausal arthritis)
4 Mode of Onset	Rarely acute usually subacute or insidious often accompanied by migratory pains	Insidious not accompanied by migratory pains
5 Patient's General Condition	Usually undernourished, anemic, and chronically ill	Well nourished frequently obese not anemic
6 Evidence of Infection	Frequently slight fever (99%) and slight leucocytosis. Foci of infection usually present	No fever no leucocytosis. Foci of infection less common
7 Joint Involvement	Symmetrical and generalized proximal interphalangeal joints especially involved	Usually asymmetrical though less generalized larger joints particularly knees but also distal interphalangeal joints involved
8 Appearance of Joints	Early Periarthritic swelling fusiform fingers Late Ankylosis extreme deformity, ulnar deviation	Early Slight articular enlargement Late More pronounced articular enlargement ankylosis slight and never complete Heberden's nodes
9 Muscular Atrophy	Often marked particularly in later stages	Not characteristic
10 Cutaneous Changes	(1) Extremities frequently cold and clammy skin atrophic and glossy redness of thenar and hypothenar eminences (2) Psoriasis occasionally present	No characteristic features Not present
11 Subcutaneous Nodules	Present in 15 to 20 per cent of cases	Normal or only slightly increased only rarely above 30 mm.
12 Sedimentation Rate	Usually greatly increased values above 30 mm in nearly all active cases	Early Slight hipping at joint margins
13 Roentgenologic Findings	Early No bony changes periarthritic swelling slight narrowing of joint spaces Late Osteoporosis bone destruction with new bone formation ankylosis and deformities	Late Marked hipping osteophyte formation and hyperostosis

the arthritic problem lies in a more universal recognition of this distinction. For the sake of clearness and conciseness the differential diagnostic points have been arranged in tabular form.

With the aid of these distinguishing characteristics, it has been possible to diagnose the type of arthritis in 95 per cent of the patients who presented themselves for treatment at the Arthritic Clinic of the Presbyterian Hospital. The majority of these differential diagnostic points are well recognized and form the basis for the classification adopted in Great Britain by the Ministry of Health and in America by numerous investigators. In this communication attention is drawn to two particular points in the differential diagnosis of the two groups.

1 The occurrence of subcutaneous nodules near the joint structures and along the course of tendon-sheaths in cases of rheumatoid arthritis. These nodules, varying in size from scarcely palpable, seed-like bodies to excrescences the size of olives, are found in 20 per cent of advanced cases of rheumatoid arthritis. Similar nodules have never been observed in cases of osteoarthritis. Histologically these nodules show a striking resemblance to those occurring in rheumatic fever. Preliminary studies on these nodules have been reported by one of us⁴ before the American Society of Pathologists and Bacteriologists and more detailed descriptions are in course of preparation.

2 The marked difference in the sedimentation reaction of the erythrocytes in the two conditions. Observations on the sedimentation rate of the red blood cells in rheumatoid and osteoarthritis are embodied in the succeeding portion of this paper. By way of further interest the sedimentation rate has been determined in 28 cases of nonarticular rheumatism ("fibrositis," "myositis," "neuritis," etc.)

II THE SEDIMENTATION RATE OF THE ERYTHROCYTES IN RHEUMATOID AND OSTEOARTHRITIS (WITH ADDITIONAL OBSERVATIONS ON 28 CASES OF NONARTICULAR RHEUMATISM)

In recent years the determination of the sedimentation rate of the erythrocytes in chronic arthritis has been recognized in European clinics⁵ as a procedure of considerable value. In America, however, this test has not received the recognition which it merits. During the past six months at the Arthritic Clinic of the Presbyterian Hospital the determination has been made as a routine procedure on all arthritic patients. Employing Westergren's modification of Fahræus' original technique, we have made approximately 500 observations on 220 patients who sought treatment in the clinic.

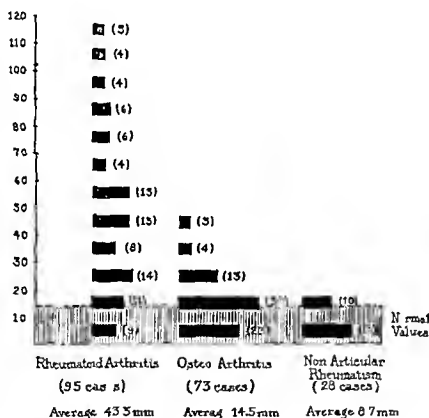
The details of the technique are as follows. Four-tenths c.c. of sterile sodium citrate (3.8 per cent) is drawn up in a 2 c.c. sterile syringe. There is 1.6 c.c. of blood withdrawn from the patient's vein in the same syringe and the mixture transferred to a test tube. A column of the citrated blood 200 mm. in height is drawn up in a 1 c.c. pipette and transferred to a rack, the base of which is formed of plasticine. After one hour the distance which the red blood cells have fallen is measured in mm. This reading is referred to as the Sedimentation Rate. The rate does not vary if the blood is drawn up into the pipette

within two hours of the venepuncture. If a longer period has elapsed there may be a slight decrease in the rate, but no great change occurs until after a period of six hours.

The results of the observations carried out on 196 patients (95 cases of rheumatoid, 73 of osteoarthritis, and 28 cases of nonarticular rheumatism) are presented in Table II. These observations were confined solely to patients suffering from chronic multiple arthritis and nonarticular rheumatism ("fibrositis," "myositis," "neuritis," etc.). The values obtained in 24 cases of related conditions, Still's disease, spondylitis, gonococcal arthritis, gout and intermittent hydrarthrosis are considered separately. As a rule several observations were

TABLE II

Sedimentation Rate of Erythrocytes
(mm in 1 hour)



made on the same patient. In all cases the highest reading obtained has been recorded. Cases with other associated conditions which might affect the sedimentation rate have not been included in this table.

A more careful analysis of the observations made in these three groups of patients reveals the additional information as shown in Table III.

In summary these observations have led to the following conclusions:

1. In active cases of rheumatoid arthritis the sedimentation rate of the red blood cells is, as a rule, greatly elevated, usually attaining values exceeding 30 mm in one hour.

2. In rheumatoid arthritis the sedimentation rate parallels to an extraordinary degree the severity and extent of the arthritic process.

3. Exacerbations are almost invariably attended by an increase, and remissions by a decrease, in the sedimentation rate.

TABLE III

	NO CASES	PER CENT
I <i>Rheumatoid Arthritis</i> —		
a <i>Values above 30 mm</i>	59	62
These cases were distributed as follows		
"Active" cases	54	56.8
"Arrested" cases	5	5.2
b <i>Values below 30 mm</i>	36	38.0
These cases were distributed as follows		
Old, arrested or "cured" cases	25	26.2
Totally cured	2	2.1
Very early cases	2	2.1
Apparently active cases	7	7.3
II <i>Osteoarthritis</i> —		
a <i>Values above 30 mm</i>	7	9.6
b <i>Values below 30 mm</i>	66	90.4
III <i>Nonarticular Rheumatism ("Fibrositis,"</i>		
<i>"Myositis," "Neuritis," etc.)—</i>		
a <i>Values above 30 mm</i>	0	0
b <i>Values above 12 mm (normal)</i>	6	21.4
c <i>Values below 12 mm</i>	22	78.5
Comparison of Averages		
a <i>Rheumatoid arthritis</i>		43.3 mm
b <i>Osteoarthritis</i>		14.5 mm
c <i>Nonarticular rheumatism</i>		8.7 mm

4 In old, long continued and arrested cases the sedimentation rate tends to return to normal values

5 In cases of osteoarthritis, on the other hand, the sedimentation rate, while as a rule slightly elevated, rarely attains values greater than 30 mm

6 All cases of nonarticular rheumatism ("myositis," "fibrositis," "neuritis," etc.) show a normal or only very slightly elevated sedimentation rate

Additional observations have been made in cases of Still's disease, spondylitis, gout, gonococcal arthritis and intermittent hydrarthrosis

Still's Disease—The readings obtained in three cases of Still's disease were as follows 75, 69, and 22. The first two of these cases were active and progressive, the third had shown definite improvement over a long period

Spondylitis—The sedimentation rate was determined in 12 cases of spondylitis. The values obtained varied from 67 to 8 but there was a very distinct difference between the readings in the infectious and noninfectious groups. Those cases that, on clinical grounds, were felt to be the result of an infectious process invariably gave higher readings than those in the osteo (hypertrophic, degenerative) group

Gonococcal Arthritis—In seven cases of gonococcal arthritis, values varying between 44 and 9 were obtained. The more acute cases invariably gave the higher readings

Gout—In one case of gout during an acute attack, the sedimentation rate was found to be 92. This observation, in conjunction with the findings of others,⁷ indicates that this test is of no value in differentiating the arthritis of gout from other types

Intermittent Hydrarthrosis—In one case of intermittent hydrarthrosis the determination gave a value of 15

In conclusion, the distinct clinical value of the determination of the sedimentation rate of the erythrocytes is clearly indicated in the differential diagnosis of rheumatoid and osteoarthritis. It must be emphasized, however, that the test should never be relied upon as the sole criterion in the differential diagnosis of the two conditions. This differentiation can usually be made on clinical grounds alone. The determination of the sedimentation time of the red blood cells usually confirms the diagnosis and contributes information of considerable prognostic value in the clinical study of the disease.

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THE SPECIFIC VACCINE TREATMENT OF CHRONIC ARTHRITIS AND RHEUMATISM

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IN THE year 1909, an unmarried woman aged thirty-five whom I had previously known as active, energetic, and healthy, consulted me. She was walking on crutches, and she was suffering from extreme pain in the dorsal and lumbar regions. She told me the following history: some twelve months previously, while abroad, she had consulted a doctor for breathlessness when playing tennis, and general lassitude. The diagnosis made was "tired heart." Rest was ordered. Severe pains in the back then developed. An orthopedic surgeon of note diagnosed tuberculosis of the spine and the patient was kept on her back in a box splint with extension, for six months. The pain grew less but recurred as severely as ever when she began to get up again. Clinically, beyond some muscular spasm there was nothing to be made out. The heart was normal. I came to the conclusion that the condition must be rheumatic. The urine was found to contain numerous streptococci. A vaccine was prepared from these in my laboratory, and after some six weeks of treatment with doses ranging from half a million to two million, the symptoms cleared up completely and have never recurred. This most dramatic case coming as it did after several other more or less successful efforts on the same lines, convinced me once and for all that the vaccine treatment of rheumatic conditions held out the best hope of curing the disease.

Since that time something over two thousand cases have been investigated bacteriologically and treated by autogenous vaccines, with steadily improving results. In 1926 I published an analysis of 490 cases of arthritis and 210 of various forms of nonarticular rheumatism.

Since that time a further 1000 cases show still better results. Not included among these are several hundred cases treated entirely by stock vaccine at the Charterhouse Rheumatism Clinic, Crosby Row, London, S.E.1, where the results are surprisingly good.

When vaccine treatment first came into vogue, it was tried for all possible bacterial diseases, but with the crude early technique many failures occurred, and as a result of a general disappointment the whole method fell rather into disrepute. This was especially the case in rheumatism and chronic arthritis, where the miraculous cures promised failed to materialize. Nor did the more sceptical hesitate to proclaim that as no improvement was to be expected in arthritic cases where bone change had occurred, the bacteriologist had no right to make vaccines and charge his unfortunate dupes the cost of such preparations, knowing full well the utter uselessness of the pro-

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cedure Even up to the present day the bulk of the medical profession remains quite unconvinced of the value of the method as applied to the chronic rheumatic diseases

To deal first with the idea that when bony change has occurred no improvement can be expected, this opinion rests on the fallacy that the symptoms are due directly to the bony change, e.g., that the pain is caused by the friction of the denuded bony surfaces. The worst symptoms of chronic arthritis are caused by inflammatory changes in and about the joint, irrespective of the amount of bony change. The pain at night, the tenderness on movement or on pressure, the intolerable aching and burning sensations are all due either to periarticular pressure, or perhaps to the granulomatous cavitation in the bones themselves. The proof of this is twofold: first, that there can be an advanced condition of osteoarthritis without any symptoms at all. Second, that notwithstanding the presence of extreme bony change, symptoms may in favorable cases entirely disappear. As an example of the first, a patient complained of pain in the left hip joint which he had noticed coming on for years. X-ray photographs showed a severe degree of osteoarthritis both in the left hip joint and in the right hip joint and from the radiographs alone it was quite impossible to say which was the worst joint. Yet the patient had never known that he had any trouble at all in his right hip.

Of the fact that the symptoms may disappear despite the severest bone change, I could give endless examples. Here is one published in my book *The Treatment of Chronic Arthritis and Rheumatism* (Oxford University Press, 1926):

"A man, aged fifty-four, suffered for three years with pain in the left hip joint, occasional pain in the left elbow and inability to sleep owing to the severity of the symptoms. He was considering throwing up his job that of a farm laborer. Treatment was started in November, 1923. It was carried on steadily until the end of June, 1925. Vaccines consisted of stock streptococci combined with autogenous vaccine prepared from the feces. Here I had found 80 per cent of streptococci of eight different varieties. He never reached very large doses, 30 million was the highest. Toward the end I was giving him only 10 million once a week. He never gave up a day's work throughout the treatment. He soon lost his pain and, except for an occasional bad night, slept well. Apart from a slight limp he now has no symptoms of any kind whatever."

The suggestion then that no improvement can be looked for in cases showing bony change is thus proved to be without foundation. Why, then, has the vaccine treatment of these cases failed so frequently as to render the medical profession entirely sceptical? There are certain very definite and quite sufficient differences in technic (both bacteriologic and clinical) to account for the poor results.

In order to understand the rational basis for the altered technic, a brief review of the successive conceptions of the mechanics of immunization may help. From the days of Pasteur up to the present, innumerable experiments have demonstrated that the effect of the injection of either killed or living but attenuated bacteria is to protect the subject from the disease caused by

that bacterium. The result is the same as that of an attack of the disease itself. Immunity is conferred. This immunity was visualized as something in the blood which killed off any hostile microbes. No doubt the rise in agglutinating, bactericidal, and opsonizing power which is demonstrable in the serum by laboratory methods, tended to crystallize this view. Certainly for many years, immunity was thought to be an affair of antibodies in the blood. The early efforts in vaccine therapy were therefore directed toward raising the blood immunity. Constantly increasing doses were found in many cases to produce a measurable rise in the antibodies. Without entirely ignoring this blood immunity, we now suppose it to be the indicator of a much deeper and more important tissue immunity. For example where a microbe, such as *B. typhosus* attacks a mucous membrane, the cells lining the intestinal wall must be the first and primary barrier, and it is these on which the immunity must have been conferred by the injection. Some confirmation of this view is to be found in the efficient immunity to many intestinal diseases which it is claimed is the result of the oral administration of bili-vaccine.

The earlier purely humeral theory influenced Koch in his treatment with tuberculin. His motto in fact was "the bigger the dose, the better the result." He had some successes, but many failures. Almoth Wright found the clue to some of these, in that he showed that the so-called tuberculin reaction was due to a local flare-up of the disease. The blood supply was increased, the toxic products from bacteria were washed out into the system, causing what he, I think, was the first to name, "autoinoculation." Wright further showed that extra blood supply, undue movement or, in lung disease, increased breathing, had the same effect as an injection of tuberculin, and autoinoculation was produced. This, if sufficiently intense, resulted in an increased area of disease. He then argued that if an injection produced autoinoculation, any successful cases were successful just because of the autoinoculation, and that therefore the right treatment must be to produce artificially, by the injection of a vaccine or otherwise, just such an amount of autoinoculation as would stimulate the system to combat the disease, but not so much as to depress the vitality of the patient so that the disease may progress. Patterson put the idea into practice. By means of carefully graded labor, he cured many tuberculous lungs under regulated autoinoculation. It was but a short step to the view that nature always cured the so-called chronic diseases by this method, and it is certainly a tenable hypothesis that in rheumatism and chronic arthritis, methods of treatment, such as radiant heat, massage, electricity, are beneficial inasmuch as they produce autoinoculation, and detrimental if and where that autoinoculation is too excessive.

In acute diseases the case is different. An invasion occurs of microbes of such virulence that the system immediately revolts. Active inflammatory changes supervene, and all the symptoms of a general and severe illness appear. But in slow and chronic disorders Wright showed by his estimations of the blood immunity that nature is not sufficiently stimulated to bring the forces of attack into play. These must therefore be artificially excited. This he asserted could best be done by the injection of what presumably was a collection of the microbes of the disease more highly concentrated than was

to be found at the site or focus of the disease, in other words, a vaccine. To summarize formerly a heightened blood immunity was the aim of vaccine treatment. Later this idea has been modified somewhat by the view that controlled autoinoculation was the main object to be kept in view. Neither of these, however, is altogether consistent with more modern views on the irritability of cells in tissues, as exemplified in its most obvious form, namely allergy. So that we require yet another conception to govern the technic of vaccine treatment. Injections of a vaccine must be so graded in strength and so spaced as to provoke just that amount of response in sensitized tissue cells which will faintly arouse their normal antibacterial powers, but that this action or reaction, if you prefer is not to be followed by a depression or slowing down of vital processes when the cells may be more susceptible to bacterial invasion. The real difference is that we now think in terms of individual cells and not of the blood immunity, or of certain tissues or organs as a whole. Apart however, from the clinical treatment side, which means the adjustment of doses and interval there are certain bacteriologic considerations which here require notice.

From all experiments and observations on vaccine treatment, it is clear that the more specific the vaccine the sharper in definition is the effect.

For successful treatment by vaccines certain conditions must be fulfilled

- 1 The actual microbes of the disease must be isolated

- 2 They must be capable of culture outside the body. One may perhaps add from personal experience that freshly isolated cultures must be used for the preparation of vaccine if it is to be thoroughly efficient.

It is necessary to examine as to how far these conditions have obtained in the vaccine treatment of rheumatism. What are the microbes of chronic arthritis? There is no doubt I think that the majority of research workers and clinicians are convinced that streptococci play a considerable part in the chronic rheumatic diseases. But it is extremely difficult to obtain convincing proof of this supposition. Whatever positive results are obtained, either in investigation or in experiments on animals there always remains some loophole through which the skeptic can escape from the inevitable conclusion. If streptococci are the cause of arthritis, then in subacute conditions they should be isolated from the blood stream. But they are seldom if ever found. In this connection one might perhaps say that the recent findings of Burbank (Bull. New York Acad. Med. 5: 176, 1929) have not been confirmed. I myself followed his technic exactly in some 50 consecutive cases, but failed to obtain a single growth of streptococci in any of them. Then again fluid obtained from infected joints is usually sterile. Cultures made from actually affected tissues round and about an arthritic joint also glands, have occasionally yielded streptococci but the results on the whole are meager and unconvincing. Nearly all published experiments lack that clear distinction of outline which is so essential to carry conviction. This is largely owing to the general haziness of our knowledge of the streptococcus group, and of our inability to distinguish the members of it or to assert with sufficient confidence that a certain streptococcus which for example, we may

have discovered from a lesion produced in a rabbit, is really the same as the streptococcus which we introduced

In 1920, the necessity for the differentiation of the streptococci became urgent for clinical reasons, I then introduced the medium now called by my name, and this together with certain standard tests, enables the bacteriologist to assure himself of the identity of any streptococcus with which he may be working, and to recognize it as and when he finds it in connection with disease in man or animals. It has frequently been urged that if autogenous vaccines are being prepared from a given streptococcus, the exact identity of the organism is a matter of but slight importance and certainly not worth an investigation, which before my method was introduced was certainly prolonged and arduous. This view is based on the idea that disease is monobacterial and that the flora underlying is fixed and immutable. My experience negatives that view. In the early days one frequently found that cases

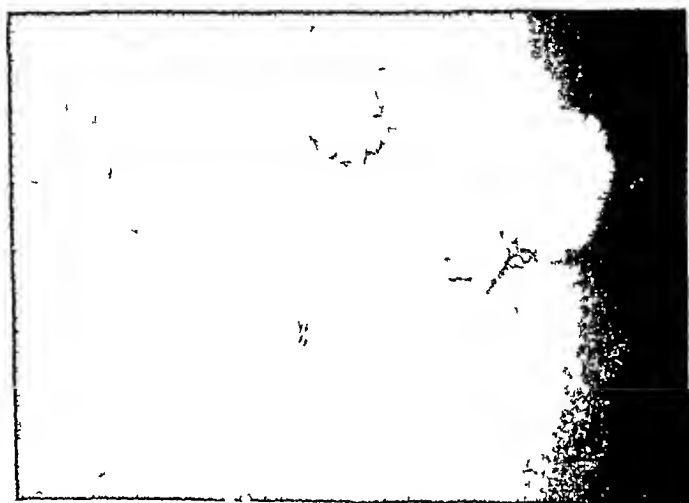


Fig 1—Primary culture on Crowe's medium from feces in a case of arthritis. Two different varieties of streptococci and a staphylococcus. Magnification by 12

of arthritis due apparently to intestinal streptococci would improve under autogenous vaccine treatment, but that relapse followed sooner or later. Further examinations then revealed that quite different streptococci were present in the bowel. Fresh vaccines were again made and used with temporary benefit. This sequence of events might occur time after time. The same was found in the treatment of chronic bronchitis. An explanation is to be found in the fact that a patient's blood will often agglutinate several different streptococci, some of which may not even be derived from the patient. Full details are given in my book.

In order to render the treatment permanently effective it seemed essential that these various latent streptococci, only a few of which were cultured at a given time, should all be contained in the vaccine. Success was achieved by combining a very polyvalent stock vaccine with the autogenous vaccine.

*The evidence in favor of the streptococcal origin of rheumatic diseases is fully set forth in the *Bacteriology and Surgery of Chronic Arthritis and Rheumatism*. H. Warren Crowe. Oxford Medical Publications. 1927.

in every case. The preparation of this stock vaccine involved the differentiation and recognition together with classification of every type of streptococcus that could be suspected of being involved in rheumatic cases. By means of photography of colonies on Crowe's medium, and a long series of biologic tests, every streptococcus isolated was cataloged to prevent duplication, and made into a separate vaccine. Eventually these were mixed together in equal amounts. The total number of different organisms was 155. This work has been described in full with all the technique in the *Bacteriology and Surgery of Chronic Arthritis and Rheumatism*.¹ Figs 1, 2, 3 and 4 will give some slight indication of the extraordinarily varied and different forms



Fig 2—Also a culture from feces showing several different varieties from a case of arthritis.



Fig 3—Primary culture from a tooth showing many different varieties of streptococci

of streptococci which can be cultured from a specimen. As an example of a relapse occurring due to reinfection by a fresh organism—a patient who had a severe rheumatoid arthritis (atrophic) after a long period of treatment made a remarkable recovery, and then went to Italy for a holiday. She returned with an extremely severe attack of muscular rheumatism. The stool culture, of which Fig 4 is a photograph, yielded what was to me an entirely new streptococcus. In the illustration are three of these "walled" colonies touching each other. Following a fresh vaccine of these organisms the rheumatism quickly cleared up.

My method of establishing the identity of a given streptococcus with rapidity and certainty, has led to what one hopes may clarify the whole of animal experiment on chronic arthritis and rheumatism. One has been able

to recognize for certain that the microbes injected into an animal are exactly the same, or different as the case may be, from those which are recovered from that animal. For example, there was found to grow from the roots of many infected teeth, whether from a case of arthritis or from a perfectly healthy person, a streptococcus which fell into my catalog under the number and letter B 7(2)h. The colony on Crowe's medium is perfectly characteris-



Fig 4—Primary culture from feces. A case referred to in the text page 1077



Fig 5—Streptococcus B 7 (2) h magnification by 1700

tic and recognizable. The sugar reactions are constant: positive to saccharose, lactose, raffinose, and negative to salicin, inulin, and mannite. Milk is always curdled within twenty-four hours. This organism has never been cultured from any other part of the body. When injected into rabbits in small doses, one cc of a washed broth culture per 1000 gm of rabbit, the rabbit usually develops arthritis in one or more of the larger joints, but

whether or not there is a clinical arthritis, the articular ends of some of the bones are in 90 per cent of animals infected by the organism. I have therefore called it osteotropic. It can be cultured from the bone and but rarely from any other tissue. It produces a granulomatous condition with bony absorption and deposition of fibrous tissue. When recovered from rabbit bone and injected into further rabbits, the process goes on indefinitely, assuming always that the conditions of the experiment are fulfilled, i.e., that the cultures are grown in a suitable medium and are freshly isolated*. Here attention might be drawn to the observations and hypothesis first put forward by Alexander Thompson (*Proc Roy Soc Med* 22 1119 1929), that osteoarthritis in the human being is essentially a disease of which the primary lesion is



Fig 6—Colonies of streptococcus B 7 () h b) 9

cavitation of bone. If that hypothesis is accepted then this condition in rabbits reproduces exactly the human disease. Figs 5 to 11 show the organism B 7(2)h, cavities in the femur of a rabbit with sections, and for comparison the same apparent lesions in man.

If the view that streptococci are the cause, or one of the causes of chronic arthritis, is correct, it will be agreed that the first condition of successful vaccine treatment is partially at any rate fulfilled when we use a polyvalent vaccine of these organisms.

In my early experience it was, however borne in on me that streptococci, important though they are, were by no means the only organism in

For details of these experiments the original paper in the *Annals of the Pickett Thomson Research Journal* Vol IX part 2, should be consulted.

volved in chronic arthritis and rheumatism. I venture here to quote a paper published in 1914 when I first put forward my suggestion that staphylococci were quite as important as streptococci in some cases of chronic arthritis. One of these which belonged to the group of the *S. epidermidis albus* (Weleh), and which I afterward named *S. epidermidis albus, variety deformatans*, was here brought forward tentatively as being the primary cause of rheumatoid arthritis (atrophic arthritis). The reasons for associating this microbe with rheumatoid arthritis seemed to me at the time extremely cogent.

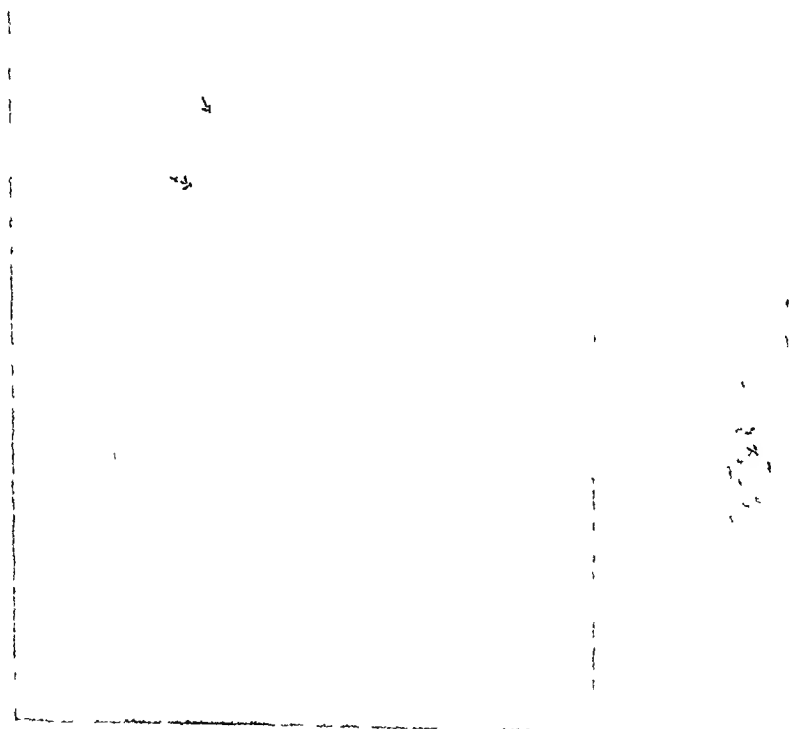


Fig 7—Antero posterior view of the knee joint of a rabbit showing cavitation in the diaphysis of the femur

“Some three years ago, from the blood of a phthisical patient, who suddenly developed acute arthritis of the left knee joint, associated with brachial neuritis, I isolated a diplococcus, which I have since learned to recognize as the organism here described, the *Micrococcus deformatans*. The case was a remarkable one, for though by no means ill, the patient (a young woman aged thirty-four years) had had for certainty three years previously a raised temperature, which on the rarest occasions dropped below 100° F. night or

morning, and usually remained about 101° F*. Never had it been found to be normal, though two daily observations had been made during the whole of the period. A vaccine prepared from this organism produced remarkable results, in that after a very few doses the knee joint became well and again serviceable, the neuritis disappeared, and the temperature dropped to normal. It was impossible to avoid the conclusion that this diplococcus was causative of the arthritis, the neuritis, and the fever. A year later, within a week of each other, I had the catheterized urines sent me of two women, both suffering from acute rheumatoid arthritis, coming on shortly after parturition. The *Micrococcus deformans* was found in pure culture and in large numbers in both cases, and in both vaccines of the organism produced that characteristic result which one always associates with the injection of a vaccine pre-

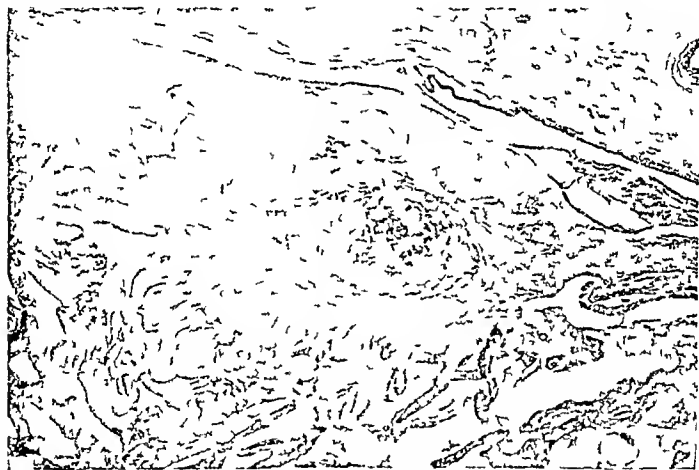


Fig. 8—Section from femoral condyle of Rabbit 11 infected with streptococcus B 7 (?) h. There is an abscess in the bone with few traces of bony trabeculae which have been removed almost in toto. Note wide area of polymorphonuclear response and new formation of fibrous tissue. Magnification 45 diameters. Stain hematoxylin and eosin.

pared from the organism pathogenic in any given case. About the same time a male rheumatoid patient consulted me. He had a chronic nasal discharge, in which the *Micrococcus deformans* was found. Still more important, it was present in pure culture in the urine. Here also the effect of a vaccine was obvious. It was already impossible to dissociate rheumatoid arthritis from the *Micrococcus deformans*."

For further evidence incriminating the *Micrococcus deformans* my books should be consulted. The evidence, large as it seems to be was, however, insufficient to carry complete conviction. Since then however further data are to hand in favor of my view.

This patient had had all sorts of vaccines up to this date without any effect on the temperature.

The blood pathogen selective test shortly to be described, has afforded valuable evidence inasmuch as the bactericidal power of the blood is found to be very low to *M. deformans* in a large number of patients with chronic arthritis. This observation is quite independent of our clinical experience, which would alone convince anybody who uses vaccines of this organism in the treatment of chronic arthritis.

So far then we have the evidence set forth very sketchily of the connection of a great many varieties of streptococci, and of the *Micrococcus deformans*, which is probably a modified skin coccus, in the causation of chronic arthritis. There may always be a third type of microbe involved. But if this is so, and clinical results are all against it, it has never yet been found. Certainly some cases are definitely infected with other types of organisms.



Fig. 9—Section from femoral condyle of Rabbit 112 infected with streptococcus B 7 (2) h. Shows abscess in the bone magnified 220 diameters. Note the typical subacute reaction with occasional giant cells.

There are several fragments of bony trabeculae in the abscess but the bone corpuscles are dead. Stain hematoxylin and mercurochrome.

B. fallax for example (Mutch). Occasionally also *B. coli* seems to be an infecting organism. Much light has recently been thrown on the systemic infection with aberrant microbes by the work of Cronin Lowe following Solis Cohen.* Pathogen selective tests whereby the patient's own microbes are

*To perform the pathogen selective test material morbi obtained from the patient is suitably diluted. Fecals are emulsified in saline and diluted until faintly opalescent. Scrapings from infected teeth are ground up in saline and centrifuged the upper portion being used for the test. Nasal mucus and sputum are treated similarly. Urine can be centrifuged and the deposit used. A microbial culture can also be diluted down for the test.

The essential feature of the test is that the patient's blood should be mixed with small amounts of the products thus prepared before it has time to clot. In practice 0.1 c.c. of the microbe containing fluid is placed in the bottom of a test tube. Five c.c. of the patient's blood freshly drawn is added. The whole is then shaken up and placed in the incubator over night. The next day the serum is planted out on a culture plate. Any organism which grows out in pure culture is then pathogen selected.

polyvalent But there is a third reason, which is that the dosage has been absolutely and entirely incorrect and given on entirely wrong principles

There are two methods of therapeutic inoculation The one attempts by frequently repeated and rapidly increasing doses to raise the blood immunity, and in the result it renders the cells of the body less sensitive to protein (microbial or otherwise), which is the cause of the symptoms The other by infrequent small stimuli has for its object the incitement of the system to keep the immunity mechanism in a state of activity Much of the difficulty and uncertainty as regards dosage and interval has arisen because of the

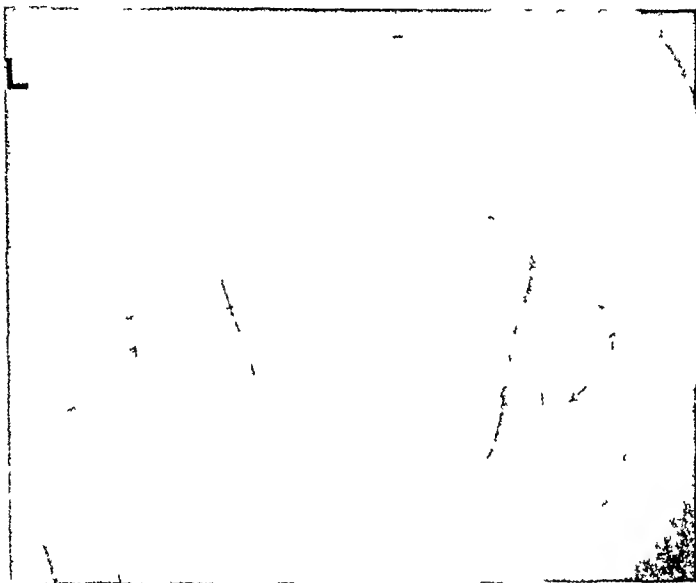


Fig 11

failure to recognize the essential difference between those conditions which are suitable respectively for the exploitation of these two methods Where the poisonous substance is extraneous to the system as in hay fever, obviously desensitization by rapidly increasing frequent doses should be the method of election, and probably equally so when the offending protein although issuing from some part of the body, produces its symptoms by its allergic action on some other part As an example, certain asthmatic paroxysms are produced by the patient's own otherwise harmless intestinal streptococci

When, however, the system is sensitive in respect of microbes which are present actually in the lesions which we are trying to cure, then the attempt

to a simple and natural classification on bacterial grounds—atrophic arthritis, hypertrophic arthritis, and mixed arthritis (when the infection is about equal and lesions of both diseases appear side by side) But the reader may ask what of menopausal arthritis, *malum coxae senilis*, metabolic arthritis dependent on endocrine, usually thyroid dysfunction In all seriousness I must state my conviction that these differ only in degree and not in kind They are all bacterial, and *equally amenable to vaccine treatment* The meno

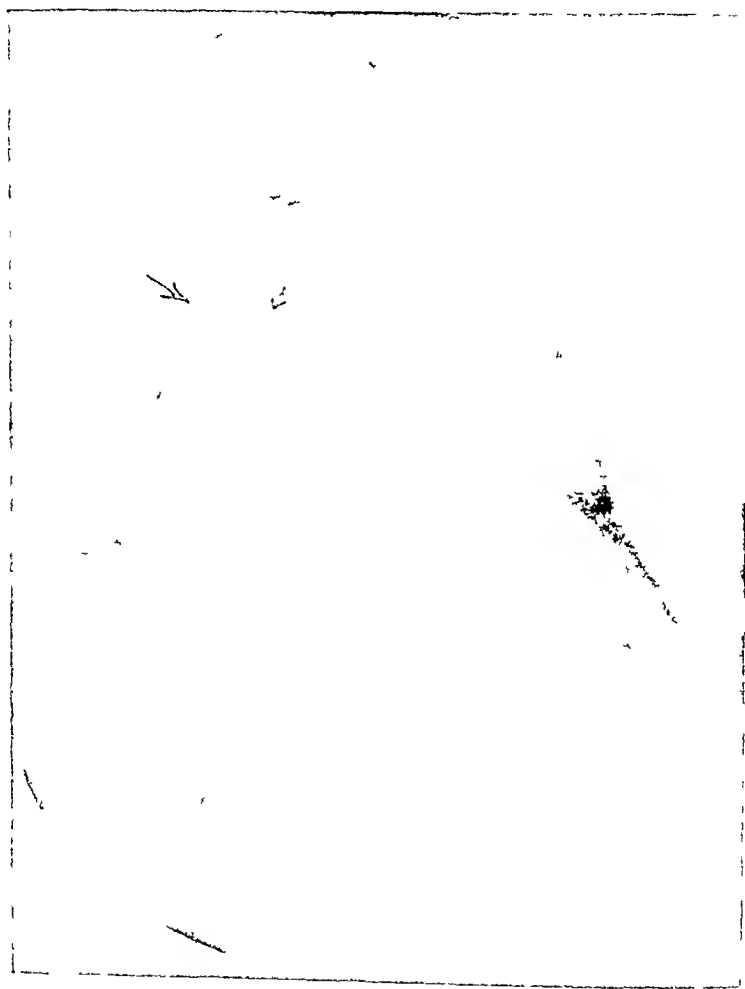


Fig 10

Figs 10 and 11—Radiographs of hip joint showing similar cavitation in man

pause is a period of lowered resistance in the first, age with or without trauma determines the second, while in the third the internal glands are themselves probably the victims of the same bacterial invasion

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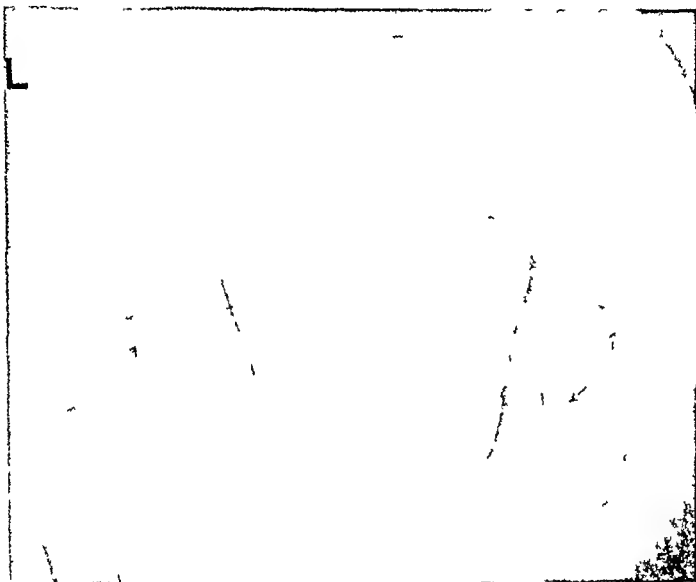


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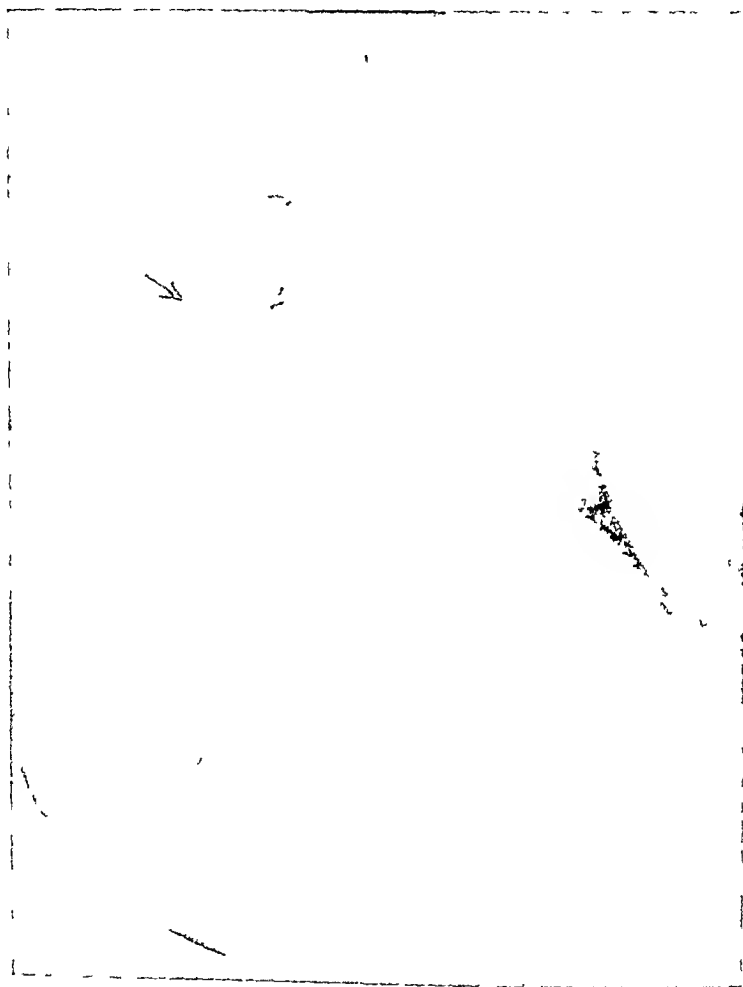


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depend on the waxing or waning of the hypothetical toxin. Even the minutest amounts might produce a disproportionate effect through the well known "hair trigger" excitability of the sensitized cells. It will be seen that the allergic hypothesis postulates first a focus of infection, the removal of which should cure the disease, and second some degree of allergy in every case. Further as a corollary, complete desensitization should always result in cure or at least definite improvement. None of these postulates can be maintained. (1) In many cases of arthritis, especially those of the atrophic type, no focus can be found, and even when present the literature of the subject is full of instances and statistics showing how frequently the removal of a focus fails to cure. (2) The majority of cases are not allergic that is to say small doses of specific antigen do not aggravate symptoms. Finally (3) desensitization does not cure and seldom improves.

(1) That a focus cannot always be found need not here be elaborated, as the fact is common knowledge among those who have studied these diseases.

(2) That the majority of rheumatic cases are not sensitive to vaccine at the beginning of treatment is a fact of experience that some become so later does not affect the question although it may render their treatment difficult.

Still it is always possible for the disciples of the allergic hypothesis to question the specificity of the antigen used, an attitude difficult to combat. In the following case one would say that the antigen must have been specific yet from the beginning of the treatment until complete cure no allergic reaction of any kind occurred.

A man aged forty six years suffering from an almost crippling degree of fibrositis (without actual joint involvement) was excreting large numbers of two varieties of streptococci in his urine. One of these was "pathogen selected." After six months treatment the symptoms had entirely disappeared and specimens of urine examined from time to time showed a steady retrogression in the numbers of bacteria until they also disappeared.

(3) Desensitization does not cure. Although the majority of cases are not in any way allergic at the beginning of treatment yet most of them show some degree of sensitiveness later on. These can sometimes be "desensitized" by pushing the dose regardless of reaction. It is a dangerous thing to do, as the lesions may rapidly extend and in any case this form of desensitization happens only through the ignorance of the practitioner. But for the purposes of argument the result of excessive dosage is valuable: the disease steadily progresses although large doses, hundreds of millions, are being injected at regular intervals.

The rival hypothesis to the allergic is much simpler and seems more tenable, viz, (1) that the lesions are due to a definite microbial invasion of the areas affected, (2) that these microbes are the "domestic" streptococci and staphylococci of the patient which may or may not be enseeded in some "focus of infection" and (3) that on account of their lower virulence, the tissues are more or less insensitive to their presence nor do the microbes usually do much harm beyond provoking some reactive fibrositis. This as Pemberton has shown, means that chill and damp by lowering the local metabolic rate, will induce twinges of rheumatism. When, however, these domestic

microbes become excessively numerous, there follows a progressive low, reactive inflammatory condition, which constitutes the condition we know as fibrositis or chronic arthritis. If the system reacts strongly to the invasion, then the condition becomes more painful and acute.

How far rheumatism is inherited remains a moot point, but on the analogy of that very similar disease tuberculosis, one would suspect that a certain type of soil was more susceptible. That "arthritis" can be "caught" is probable and may account partly for familial recurrence. A clear case recently came up for treatment when a woman had for two years nursed a case of severe arthritis, and then although up until that time nonrheumatic, she was developing exactly the same type of lesions as her patient.

The technic of the treatment of chronic arthritis by vaccines, with which this article is concerned, is based on the simple hypothesis of the direct invasion of the tissues by the microbes of the disease, that these microbes are streptococci of many different varieties, but always *nonhemolytic* varieties, and staphylococci of one or at most two types while occasionally but rarely other infecting organisms may play a part.

I am not here proposing to do more than sketch out in very rough outline the treatment in practice, as this is fully described in my *Handbook on the Vaccine Treatment of Chronic Rheumatic Diseases* (Oxford Medical Publications, 1930). In this book, three alternative methods are given. The first is that in use as a general routine at the Chatterhouse Rheumatism Clinic, where stock vaccines alone are used, and patients are seen and injected once a week. The second is somewhat simpler and is also suitable for large scale work, whereas the third is more elastic as befits private practice, where autogenous vaccines are prepared after full bacteriologic examinations.

The reader will have gathered already that the principles underlying the method here put forward are entirely opposed to those ordinarily followed. The idea that "the bigger the dose the better the result" must be entirely discarded. Our object is not to attempt to produce a "solid immunity" but to stimulate the individual tissue cells by the smallest possible effective doses, to bring into action their antibacterial mechanism, without subsequent depression of that mechanism. Since the effect of a general "reaction" is temporarily at least to depress the antibacterial activity of the whole system, such reactions are to be avoided. It is therefore very necessary to get a clear picture of what is meant by reaction. Reaction is of three distinct kinds: local, focal, and general. The first of these practically never occurs with streptococcus or *M. deformans* vaccine if the injection is given subcutaneously. A focal reaction is indicated by increased symptoms in the disease area, e.g., pain or swelling in a joint, muscular spasm, and the like. Unless a focal reaction is excessive, resulting in stasis or acute exacerbation, it does not do much harm, but should be avoided as being unpleasant to the patient, and unnecessary to the "cure." A general reaction on the other hand must be avoided at all costs. Yet a general reaction may be far less irksome and only noticeable if a careful watch is kept. The signs vary from severe malaise with headache and temperature to a mild feeling of lassitude or sleepiness, or even only a period of lag before improvement sets in. If a patient is disinclined

for his usual activities or admits to being slightly drowsy within forty eight hours of the dose, he is suffering from a general reaction. When this occurs rest is essential and therefore the doses should always be given on such days as will admit of rest on the following day if necessary. Except where general reaction occurs, no change in the patient's activities or way of life is necessary or in fact desirable. Then the idea must be discarded that the actual size of the dose matters in the least, as long as it is the best dose for the patient. It may be lower than 1000 organisms or as high as 1000 million! But much more probably nearer the former. The majority of cases progress steadily on doses ranging from 1000 to 100,000 and but few require more than the higher of these two, except in the very early stages when the majority of patients are insensible. During treatment at least 90 per cent of cases develop some degree of sensitiveness. It is this fact which constitutes the main source of failure. It explains the very frequent statement by patients that vaccine treatment did them good to begin with, but that afterward it seemed to make them worse. At the first hint of the development of sensitiveness the dose must be drastically reduced and the greatest caution exercised both in size of dose and length of interval. In practice, especially in the earlier stages of treatment, one should make it a fixed rule to reduce the dose to one tenth of that which is followed by *any sign whatsoever* of a general reaction. A focal reaction demands a drop to half, or if severe to one fifth, of the exciting dose. During the earlier stages of sensitiveness, doses should not be given more often than once a week, and sometimes a longer interval is even better, especially if the effect of the dose is ambiguous.

When a case of chronic arthritis comes up for treatment, circumstances will decide whether autogenous vaccine is to be given or stock. We will consider an hypothetical case, where full bacteriologic facilities are available, since the actual scheme of dosage is the same.

At the preliminary interview, after entering up the history and result of examination, arrangements must be made for dental radiograms and the collection of specimens of urine (catheter in female) feces (after a purge) posterior nares (if catarrh), etc. Also a tooth must be extracted for culture purposes if dental sepsis is present*. Pathogen selective blood tests are also made. It is advisable to start the treatment by some doses of stock vaccine to test the tolerance of the patient. In this way also no time is wasted. The treatment should begin with an injection of 100,000 polyvalent streptococcus vaccine, five days to a week later 100 000 M deformans† is given. There must follow from one or other or both of these doses (a) a focal reaction, (b) a general reaction, (c) definite improvement, or (d) no change whatsoever. There are now two distinct lines of advance. (1) To carry on alternating the streptococcus and M deformans vaccine until we find the reaction point of *each* and then to combine the two together in just that proportion or (2) to

For all the details of the bacteriologic and other technic reference can be made to my books

†The vaccines used by the author can be obtained from Messrs Reynolds & Branson Ltd 13 Briggate Leeds

combine them at once in equal quantities. The amount of the dose is then regulated entirely by the dose of that organism to which the patient is most sensitive. The second method is to be preferred in all cases at the beginning of treatment. Later if difficulty arises the two kinds of vaccine can be tested out separately, but in *no case* must either be entirely omitted.

What now should be the third dose? Let us consider the contingent effects of the first two doses, the following schedule will give the correct procedure

- a (focal reaction) following either first or second dose,
 if slight give { 50,000 streptococci
 50,000 M deformans
 if severe give 20,000 āā
- b (general reaction) following either first or second dose,
 whether slight or severe give 10,000 āā
- c (definite improvement) following either first or second
 dose, give 100,000 āā
- d (nil) following either first or second dose, give 100,000 āā

After the third dose the autogenous vaccine should be ready and must then be combined with stock vaccine (the latter must never be omitted). Again we assess the next dose from the same considerations as before and so on while the treatments last. Be it particularly noted that the amount of each dose depends on the effect of the preceding dose and on *that alone*.

TABLE FOR THE FOURTH AND SUBSEQUENT DOSES

EFFECT OF FIRST OR SECOND DOSE	THIRD DOSE	EFFECT	FOURTH DOSE
(a) slight	50,000 āā	(a) slight	25,000 āā
		severe	10,000 "
		(b)	5,000 "
		(c)	50,000 "
		(d)	75,000 "
(a) severe	20,000 āā	(a) slight	10,000 "
		severe	4,000 "
		(b)	2,000 "
		(c)	20,000 "
		(d)	30,000 "
(b)	10,000 āā	(a) slight	5,000 "
		severe	2,000 "
		(b)	1,000 "
		(c)	10,000 "
		(d)	15,000 "
(c)	100,000 āā	(a) slight	50,000 "
		severe	20,000 "
		(b)	10,000 "
		(c)	100,000 "
		(d)	150,000 "
(d)	100,000 āā	(a) slight	50,000 "
		severe	20,000 "
		(b)	10,000 "
		(c)	100,000 "
		(d)	150,000 "

The following is an example in practice of the result of drastic reduction of dose. A patient suffering from a very severe and intractable brachial

neuralgia, with slight nerve tenderness (some neuritis), was quite unable to work. Extreme pain at night prevented sleep. Treatment: stock vaccine once a week.

First dose 100,000 streptococci	Pain severe, sleepy, sick (b)
Second dose, 100,000 m. deformans	Tired and drowsy (b)
Third dose 10,000 aa	No improvement out of sorts, pain as before (b)
Fourth dose, 1,000 aa	Pain disappeared almost entirely twenty-four hours later. Be- ginning to recur after six days
Fifth dose 1,000 aa and so on	Result excellent

Compare these doses with those usually sent out by commercial firms, or recommended by well-known authorities. Pemberton, in his last book, gives up to 2000 million at intervals of five to seven days.

Apart from the results of treatment which show that small doses are quite efficient, further justification is to be found in a comparison with the modern tuberculin dosage. Those who give unaltered tuberculin—that is, the bacillary emulsion, use a range of from one to ten millionths of a milligram in children and perhaps five to ten times that dose in adults. The equivalent dose of the cocci which constitute the vaccine for rheumatism would be for children from one to ten thousand and for adults from ten to one hundred thousand.

Occasionally patients develop during treatment such a marked degree of sensitiveness that even 1000 organisms are not tolerated. Various methods are under trial for dealing with the situation. The simplest is to use benzamine lactate with the vaccine. It is then rendered more tolerable to the patient. A 4 per cent solution of benzamine lactate should be put up in sterile vaccine bottles. This must be combined with the vaccine in such amount that the whole solution injected contains not less than 2 per cent of the drug. It is only necessary to use it where doses of less than 1000 are followed by violent reactions. The vaccine itself should be put up in as small a bulk as possible, so that not more than a total of half a c.c. of 4 per cent benzamine lactate is required.

The rationale of this peculiar character is obscure. Possibly the excitability of the tissue cells is lulled by the anesthetic properties of the drug. Other methods of dealing with the sensitive state are described in my previous books.

The length of the treatment depends on the progress of the 'cure' and is extremely variable. There is no fixed term or definite number of doses which constitute a 'course'. One will keep in mind the general principle that, so long as the injections are found necessary to prevent relapse, they should be continued.

The matter is easily put to the test. The patient is merely directed to wait until symptoms tend to recur before coming back for his dose. In time it will be found that even with quite small doses, once a fortnight will be

sufficiently often to keep down all signs of rheumatism, and later that for three weeks or longer no injection is required

The age of the patient has a considerable bearing on the question. Young people, for example, will throw off the infection rapidly and completely unless there is some active focus of infection present. This must be suspected if the patient exhibits undue sensitiveness. Older patients, on the other hand, and those in whom the disease has been of long standing, may never become quite free, and will require a few doses once or twice a year.

In conclusion, I would beg to advance the plea that vaccine treatment should not be used only and as a last resort in advanced arthritis and rheumatism, where crippling deformities may prevent complete functional cure, but that the method should also be adopted in early cases when it is almost invariably successful.

15 PORTLAND PLACE.

THE BIOLOGIC PRODUCTS OF STREPTOCOCCUS CARDIOARTHRI- TIDIS AND THE LATTEST DEVELOPMENTS IN THE TECHNIC OF THEIR THERAPEUTIC APPLICATIONS

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IT HAS been nearly four years since the biologic products of Streptococcus cardioarthritidis were introduced in the treatment of rheumatic fever.¹ This period has provided abundant opportunity for observing patients in whom these products have been used during this initial stage in their development. It appeared rather early that the antiserum did not represent a finished therapeutic agent which could be used successfully in treatment unless certain imperfections of it were appreciated. A technic in its application had to be developed with a view of minimizing the untoward effects likely to arise because of these imperfections. This technic differs in several important particulars from the usual technic of antiserum therapy.

The attempts to supplement the treatment with antiserum with that of a bacterial vaccine prepared from Streptococcus cardioarthritidis very early revealed the dangers of using such a vaccine in dosages commonly employed in vaccine therapy, and after repeated reductions in dosage of the vaccine, eventually led to the preparation and use of a normal saline extract of the streptococcus. This product designated a soluble antigen of Streptococcus cardioarthritidis is a new and very potent agent in biologic therapy. The attempts to develop a technic in its application which would be most efficient have continued since January, 1928. The result has been that much has been learned of the unusual characters of this product, and the technic in its application has been developed after much trial and error. This technic is not to be regarded as perfected so that those who undertake to use this agent in treatment should be ever alert to any future developments and ever ready to modify their methods accordingly.

Since both the antiserum and the soluble antigen of Streptococcus cardioarthritidis are being used rather widely and after methods which do not take into account the more recent developments in the technic of their applications,² it is deemed important to record what our intensive study of these agents has revealed to date of their properties and of the most effective methods in their application.

THE "FOCAL REACTION" FOLLOWING LIBERAL AMOUNTS OF THE ANTISERUM OF STREPTOCOCCUS CARDIOARTHRI- TIDIS AS AN EVIDENCE OF ITS PRESENT IMPERFECTION

In using the antiserum, a most important consideration is that of avoiding certain deleterious effects associated with the employment of dosage which is too liberal in amount. This has been designated the "Focal Reaction"³ and described in early publications. Its harmful effects are particularly observed in

patients acutely and gravely ill with rheumatic fever, who are also the patients in whom one is inclined to employ the largest amounts of antiserum. The outstanding clinical features of the "focal reaction" are an actual extension of the acute arthritis, a marked increase in the leucocyte count, and a continued elevation of temperature. The extensions of the acute arthritis are particularly prone to involve the small joints of the fingers which become swollen and the skin over them reddened. Usually the pain and tenderness is much less than one would expect from the angry appearance of the joints. With this reaction the leucocyte count is very markedly elevated, counts of 30,000 to 40,000 per mm are common in patients who showed counts of 15,000 to 20,000 prior to the onset of the reaction. A leucocyte count as high as 70,000 has been observed in this reaction. The temperature during the focal reaction may rise slightly above the high points recorded prior to its onset but does not tend to show the remissions ordinarily so characteristic of rheumatic fever except toward the end of the reaction period when daily fluctuations are noted. Very careful discrimination and some experience with the more severe forms of this reaction are necessary in order to differentiate it from the spontaneous extensions of the disease. Severe reactions may last for twelve to fourteen days, but a period of from five to eight days is more common. They may be terminated promptly by adequate administration of salicylates for one or two days only and the symptoms do not tend to recur after this medication is discontinued abruptly.

This type of reaction is not prominent following the use of antitoxic serum in diphtheria and tetanus. A theoretical explanation of it³ has been suggested based largely upon the facts developed by Swift and his coworkers⁴ which have demonstrated that certain protein products of streptococci are potent antigens for inducing hypersensitive states in animals. Since hypersensitive states induced by bacterial protein can be transferred passively,⁵ it was suggested that the "focal reaction" in patients treated with the antiserum of streptococci represented the aggravation of certain allergic symptoms of the disease which was brought about by the transfer to the patient of the antibodies of these protein factors of streptococci contained in the antiserum used for therapeutic purposes. It was pointed out further that this condition might also be expected to apply in cases of other antistreptococcal sera prepared by the method of injecting animals with the whole bacteria rather than with their toxins only. The antiprotein antibody would appear in an antiserum as a separate and distinct antibody from any antitoxin. The former is not species specific, the latter is species specific. The removal of the antiprotein antibody without affecting the species specific antitoxin content of such a serum has been suggested as the outstanding requisite³ for perfecting it as a therapeutic agent. We believe, therefore, that before the specificity of any antistreptococcus serum can be established beyond doubt in rheumatic fever, this separation of antiprotein antibody from the bacterial species specific antibodies must be effected.

The following selected case history presents many of the features of a severe "focal reaction" which was allowed to run its course without salicylates. Morphine or codeine was used as required to provide relative comfort for the patient.

CASE 1—J W Admitted Dec 31, 1927, discharged Feb 1, 1928 Colored female, aged twenty four years, domestic Diagnosis acute rheumatic fever

Following an acute upper respiratory infection, this patient began six days before admission to have pain in the right hip, and pain, swelling and tenderness in the left ankle, left foot, the right ankle, and the knees She had no previous attacks similar to this but three years ago she had pain and stiffness in the right hand and wrist lasting only three or four days She has had "colds" frequently and "sore throat" occasionally At the time of admission there was pain, swelling and tenderness in both knees and stiffness in the right hip The patient appeared toxic, and there was profuse sweating The conjunctivae were moderately injected, the tonsils were enlarged and the whole pharynx hyperemic The submaxillary glands were enlarged but not tender The pulse rate was 120 per minute, and the temperature was 101.2 F The pulmonary areas were clear The cardiac apex impulse was in the fourth interspace and 10 cm to the left of the midsternal line There was a palpable thrill over the apex, and a rough systolic murmur over the mitral area There were no signs of cardiac decompensation The liver and spleen were not enlarged or tender

Laboratory Findings—

- Blood count leucocytes 11,000 per c.mm
- Blood culture sterile
- Blood urea nitrogen 17 mg per 100 cc blood
- Blood Wassermann cholesterinized antigen plus four acetone insoluble antigen negative
- G C complement fixation, negative

The patient received by intramuscular injection 20 cc of the concentrated equine antiserum of *Streptococcus cardioarthritidis** on Dec 31, 1927 and an additional 20 cc on Jan 1, 1928 Salicylates were not administered Codeine was used as required for symptomatic relief

The summary below presents daily records of the condition of the joints the maximum and minimal pulse rates and temperature readings the opsonic index for *Streptococcus cardioarthritidis* of the patient's serum and the leucocyte counts The extension of the acute arthritis the continued elevation of temperature without the characteristic daily fluctuations and the marked increase in the leucocyte count are characteristic of the focal reaction which in this case lasted for about ten days

THE TECHNIC OF TREATMENT WITH ANTISERUM OF STREPTOCOCCUS CARDIOARTHRITIDIS

With this imperfection existing in the antiserum of *Streptococcus cardioarthritidis* recourse was taken to methods in its clinical application which, until such time as the imperfection of the serum could be eliminated would be helpful in preventing the more severe forms of the "focal reaction"

The procedures found to be of greatest value were

- 1 A reduction of the dosage of antiserum to the estimated minimal requirements of the patient
- 2 The administration of the estimated total dosage in divided amounts allowing an interval of eighteen to twenty four hours between the several injections
- 3 The use of moderate doses of salicylates for the few days only during which the antiserum was being administered

This technic of treatment with antiserum differs rather radically from the usual procedure in the administration of a therapeutic antiserum—the keynote of which is prompt administration of liberal dosage Such should also be the

*The biologic products of *Streptococcus cardioarthritidis* used in the patients cited in this communication were provided through the courtesy of the H. K. Mulford Company of Philadelphia

TABLE I
SUMMARY OF CASE 1

DATE	CONDITION OF JOINTS			PULSE RATE	OPSONIC INDEX	TEMPERATURES		SIRUM TREAT	SIRUM DISFAST	IFUCOCYTE COUNTS
	PAIN	SWELLING	FINDINGS			MAX	MIN			
12/31/27	BK	BK	BK	90-120		101.2	100.2			11,200
1/1/28	BK	BK	BK	96-110		102.0	99.8	20 cc conc		
1/2/28	BK	BK	BK	96-110		102.6	99.8	20 cc conc		
1/3/28	LK	BK	LK	90-110	12	102.4	101.0			14,000
1/4/28	RE	RW	RE, RW, LK	110-124	08	103.2	101.0			20,000
1/5/28	LI	LI	LI	100-112	07	103.4	100.6			11,000
1/6/28				96-110	09	101.0	100.6			12,200
1/7/28	LW, LE	LW, LE	LW, LS, LE	90-110	12	101.4	101.6		+	8,600
1/8/28	LW	LW	LW	112-120		102.4	102.0		+	
1/9/28	RE, RW	RW	RW, RE, BK	110-130	11	102.8	101.6		+	19,800
1/10/28	RW	RW	RW, LK	120-140	09	103.2	100.0			18,100
1/11/28	BW	BW	BW	120-130		102.6	100.2			10,600
1/12/28	BW	BW	BW	110-110		102.6	100.2			
1/13/28	LW	BW	LW	112-134	06	102.2	99.6			
1/14/28	LW	BW	LW	114-130		101.6	99.4			
1/15/28	LW	BW, RW	LW	100-130		102.1	99.0			
1/16/28				104-120	09	103.4	99.2			
1/17/28				110-120		98.6	98.4			

B K both knees L K left knee R E right elbow R W right wrist L D left elbow L W left wrist L S left shoulder

method of choice in the use of antistreptococcal serums if it were not for the fact that in their present stage of development they are prone to set up the undesirable 'focal reactions' and therefore cannot with expediency be used in as liberal amounts as would be most desirable. This serves to emphasize the twofold nature of the problem—that of perfecting an antiserum and that of developing a technic of its application. The latter is most important in the present stage of development of this antiserum and may be outlined briefly as follows:

1 The total dosage recommended in the treatment of a patient with acute rheumatic fever is 5 c.c. of the concentrated equine antiserum per one hundred pounds of body weight except when the patient weighs less than one hundred pounds when 5 c.c. is regarded as the minimal effective dosage. With the unconcentrated hovic antiserum a volume dosage of 20 c.c. is regarded as the equivalent of 5 c.c. of the concentrated equine antiserum.

2 The antiserum is injected intramuscularly (never intravenously) using 40 per cent of the total estimated dosage as the first injection, which is followed after eighteen to twenty-four hours by a second injection of the remaining 60 per cent. Occasionally where a partial response only is adjudged as being obtained following the second injection, a third injection equal in amount to that of the second may be practiced on the third, or fourth day, but no injections thereafter are recommended.

3 Sodium salicylate, or its equivalent in related compounds, is administered in daily amounts of sixty to eighty grains from the beginning of the administration of serum and continued until twenty-four hours after the last injection of antiserum, whereupon it may be discontinued abruptly.

The following selected case history represents a patient in whom small doses of antiserum were used. Salicylate medication was not used in this patient in conjunction with the serum treatment. From the history, this patient had been receiving salicylate medication up to the time of admission to the hospital, and it is probable that this may account for the absence of leucocytosis on admission.

CASE 2—F. R. Admitted April 10, 1928, discharged May 4, 1928. White, male, aged nineteen years, baker. Diagnosis: acute rheumatic fever.

Two weeks prior to the onset of the acute arthritis of rheumatic fever this patient suffered an attack of acute upper respiratory infection which was diagnosed 'la grippe'. He had never had a previous attack of rheumatic fever but had suffered occasional attacks of tonsillitis. The acute arthritis first appeared in the right ankle, and later involved the left ankle, and both knees.

At the time of admission the patient was suffering from acute pain in the right wrist, left knee and right ankle. These joints were swollen, very tender, and the skin over them was reddened. There was also tenderness and pain on motion in the left wrist, right knee, right elbow and right shoulder. The patient appeared very toxic. The skin was moist and pallid. The pulse rate was 100 per minute and the temperature 102° F. The tonsils were enlarged and cryptic but not acutely inflamed. The lung areas were clear. The cardiac apex was in the fourth interspace, 9.5 cm. from midsternal line. The right border was at the right sternal margin. A slight thrill was noted over the apex as was also a soft systolic murmur, transmitted to the left axilla. The second sound at the pulmonary auscultation area was accentuated. There was no tenderness over the abdomen. The liver and spleen were not palpable.

Laboratory Findings—

Urino Amber color, acid, specific gravity 1.030, no albumin, no sugar, no casts, epithelial cells and a few leucocytes noted

Blood count Hemoglobin 14.9 gm per 100 cc, erythrocytes 4,700,000, leucocytes 7,300, polymorphonuclears 68 per cent, lymphocytes 32 per cent

Blood urea nitrogen 12 mg per 100 cc blood

Blood uric acid 3.6 mg per 100 cc blood

Blood sugar 133 mg per 100 cc blood

Blood culture, sterile

Throat culture, *Streptococcus cardioarthritidis*

Blood Wassermann, negative

Patient's serum up to dilution of 1:80 agglutinated suspensions of *Streptococcus cardioarthritidis*, and the opsonic index with this streptococcus was 0.5

This patient received by intramuscular injection 5 cc of the concentrated equine antiserum of *Streptococcus cardioarthritidis* on April 10, 1928, and an additional 5 cc on April 11, 1928, or a total dose of only one fourth that used in Case 1.

The progress record (Table II) contrasts sharply with that of Case 1, in that a "focal reaction" did not develop. The prompt rise of the opsonic index for *Streptococcus cardioarthritidis* with the clinical improvement is characteristic of the value of this test in practice.

THE DEVELOPMENT AND USE OF AQUEOUS EXTRACTS OF *STREPTOCOCCUS CARDIOARTHRITIDIS*

Appreciating the persistent character of the lesions of rheumatic fever as they occur in the blood vessels and the heart, it is scarcely to be expected that any antiserum which at best confers protection over a period of weeks, would alone constitute an adequate treatment. It appeared desirable to attempt to supplement any passive protection conferred by antiserum, by inducing, if possible, an active immunity from the use of bacterial vaccines, or products of a related nature. It also was regarded as important to begin these attempts as soon as appeared feasible after the use of antiserum in order to induce some degree of active immunity before the period of passive protection provided by antiserum had terminated.

With bacterial vaccines as ordinarily employed difficulties were encountered from the very beginning. Relapses apparently associated with the injections of vaccine occurred with doses as small as 10,000 of the devitalized streptococci. This unfavorable experience very early led to the development of aqueous extracts of the bacteria in order that dosage might be still further reduced. Thereafter we met with failures intermixed with apparent success through a period during which dosages were being constantly reduced, until the present time when such minute amounts of the aqueous extract of *Streptococcus cardioarthritidis* are being used that they can scarcely be regarded as capable of inducing an active immunity after the manner of a bacterial vaccine. Our best results with these aqueous extracts of streptococci are our latest results. After working with them and studying carefully the responses of many patients over a period of more than two years, it is more than ever apparent that we have only touched upon their possibilities in therapy and further, that the dosages and methods of administration as developed to date do not represent perfection. The present dosages and the technique of their employment are safe and yield therapeutic results which cannot be obtained with bacterial vaccines but we do

feel that more prompt and striking results with these extracts are yet to be obtained upon learning more of the manner in which they act and upon further development of the technique in their application

The four dilutions of these aqueous extracts of *Streptococcus cardioarthritidis* now in use contain respectively per cubic centimeter, the water-soluble materials of 0.1, of 1, of 10, and of 100 streptococci. They have been designated soluble antigens of *Streptococcus cardioarthritidis* and the dilutions above mentioned are referred to as 1:1 billion, 1:100 million, 1:10 million and 1:1 million respectively. Our experience in the beginning with dilutions of 1:10, 1:100, 1:1000, 1:10,000, and 1:100,000 was unsatisfactory, in that activation of symptoms frequently occurred while patients were under treatment with subcutaneous injections of amounts ranging from 0.05 cc to 1.0 cc. Wilson² in a recent article records a few patients in whom she employed the soluble antigen of *Streptococcus cardioarthritidis* in dilutions of 1:100 and 1:1000. These dilutions were those used for a brief period very early in our work but are stronger than those recommended in my first publication on the use of antigen in May, 1928, and much in excess of recommendations issued in form letters of December 12, 1928, to those to whom the antigen was furnished for clinical trials, when the dilution of 1:100 millions was already arrived at in the development of this method of treatment. The high percentage of relapses in her patients while under treatment with the dilutions used serves to indicate the danger attending the use of excessive dosage and demonstrates how readily one may nullify the effects of antiserum by a supplemental course of the antigen employed in dosages which are too large. Yet these disastrous effects of antigen are so subtle that even such a capable clinician as Wilson failed to incriminate it as playing a part in the relapses noted in her cases. The reasons for this will appear under the discussion of the reactions following the use of the soluble antigen.

REACTIONS FOLLOWING INJECTIONS OF SOLUBLE ANTIGEN OF *STREPTOCOCCUS* *CARDIOARTHRITIDIS*

As between the antiserum and the soluble antigen of *Streptococcus cardioarthritidis*, the latter is by far the most difficult to apply clinically. Progress in its development for clinical use was made only through trial and error. The errors have been largely from the use of overdosage. This will be readily understood when it is appreciated that the adjustment of dosage downward soon reached amounts so minute as to be entirely new in any form of biologic therapy. It is very difficult to conceive of any effect whatever as being produced in patients by a fractional part of the extract of a single streptococcus and yet the extensive use of the extract has amply demonstrated that very marked reactions in patients with rheumatic fever, and in patients with chronic atrophic and hypertrophic arthritis, may follow injections of such minute amounts. Reactions following dosage as minute as this suggest an extremely hypersensitive condition on the part of these patients.

The fact that patients with chronic arthritis appear as hypersensitive to this product, as do those with rheumatic fever has favored its study in patients by providing an abundant supply of clinical material in which a standardiza-

tion of dosage could be practiced with safety. Such procedure is not practical in patients with rheumatic fever because of the dangers of activating the serious cardiac lesions by overdosage. A further advantage in chronic arthritis, especially in the atrophic type, was provided in the superficial character of the lesions in the joints so that any activation or subsidence of the inflammation could be studied objectively in them.

A disadvantage in using this type of patient for study arises because of the spontaneous activation or subsidence of signs of inflammation about the joints under a great variety of influences entirely beyond experimental control. It has therefore been necessary to study the clinical effects of injections of this antigen in a very large number of patients. Based on experience of this kind the following may be stated as our experience to date.

The reactions following the injections of antigen may be classified in order of their frequency of occurrence, as

I Focal II General III Localizing IV Local

I The focal reaction occurs constantly with proper dosage. It consists of an activation of both the objective and subjective symptoms attending the joints involved in the arthritis. Frequently joints which have been quiescent for months will show activity as well. Myalgias and neuralgias are also frequent manifestations in this type of reaction. The most severe focal reactions occur with the secondary phase of the general reaction described below.

II The general reaction is biphasic when excessive dosage is used, but with the smaller more properly adjusted dosage, the primary phase of the reaction is not noted, only the secondary phase appearing. The primary phase usually appears within the first twelve to eighteen hours and may continue from six to twenty four or more hours. Extreme lassitude and malaise with drowsiness followed by wakefulness are outstanding manifestations of this phase of the general reaction. The tendency is for the blood pressure to be reduced, and the pulse to be accelerated. A slight rise of temperature occurs at times in afebrile patients and more commonly in febrile patients. Little change or only a slight aggravation of the arthritis occurs with this phase. This phase is followed by a period of euphoria which may extend over two or three days during which the patient experiences relative comfort in the joints and is greatly encouraged. Following this, the secondary phase comes on gradually with a return of all the arthritic symptoms and characterized especially by marked tremulousness, nervous excitability, emotional depression, irritability, anorexia, nausea and occasionally vomiting, a cool moist skin, slow pulse, lowered blood pressure, and a tendency to a subnormal temperature.

The primary phase of the reaction is aggravated by the injection of more antigen and is associated with overdosage. The secondary phase of the reaction is promptly alleviated by the injection of more antigen. This relief occurs within two hours after subcutaneous injections, and has been observed to appear eight minutes after intravenous injections. The primary phase of the reaction should never be produced with dosage properly adjusted to the patient and the secondary phase should be anticipated by a repetition of the injection. This ideal condition constitutes the greatest difficulty in practice because we have

at present no means of anticipating how the patient will react and each patient must be subjected to trial with minute dosage, and adjustments made as soon as possible upon observing his reactions. For the best clinical results these adjustments should be made as speedily as possible to a dosage so small that the patient will be maintained either in the euphoric stage or, better still, to a dosage so small that only the mildest symptoms of the secondary phase of the reaction appear within several hours after the injection. Certain peculiarities arise here which have not been met in any other form of biologic therapy, and should be emphasized even though they cannot be explained in the present state of our knowledge.

Gross overdosage with antigen does not increase the severity of the symptoms of the general reaction, it merely delays their appearance and prolongs the duration of the several phases of the reaction. A patient may continue in the primary phase of the reaction, which is very difficult to recognize as such, for from eight to fourteen days following a single injection of, for example, 0.05 cc of a 1:1000 dilution of antigen and without any period of euphoria, before the easily recognized secondary phase appears, whereas, 0.05 cc of a dilution of 1:100,000,000 may produce no symptoms of the primary phase but an immediate period of euphoria lasting from four to eighteen hours after which a very sharp and stormy secondary phase will appear. In the first instance the patient without further treatment may continue for two or three weeks in an active cycle of arthritis before it subsides to the preinjection level, in the latter case, the symptoms of the secondary phase may continue for a few days only, after which the arthritis may or may not be less troublesome than before the injection. It is difficult to associate any reaction with the larger dosage because the symptoms appear so late and come on gradually. The reactions following the smaller dosage are more easily associated with its injection because they come on more promptly and the symptoms are more stormy in character. This condition of affairs constitutes an apparent paradox which has been discussed from a theoretical standpoint in a recently advanced hypothesis.³

A patient upon being started in this treatment with a very minute dose may show neither a primary phase nor a period of euphoria, but an immediate reaction of the type of the secondary phase. This has been observed to come on within fifteen minutes after an intravenous injection of 0.1 cc of a special dilution of 1:100 billion and to last for three and one-half hours when the symptoms of it terminated very abruptly. While the very unusual character of these responses cannot be explained, the facts observed in practice are clear and may be restated in the generalizations:

1. Large doses of antigen are followed by indefinite reactions, greatly delayed in their appearance and very prolonged in duration.

2. As the dosage is regulated downward the time elapsing between its injection and the appearance of the primary phase of the reaction is shortened, then this phase fails to appear, being replaced immediately by the period of euphoria and lastly the period of euphoria fails to appear but instead a prompt and sharp reaction of the type of the secondary phase. Following repeated injections of the infinitely small amounts of antigen eliciting the latter type of reaction, the symptoms on each repetition grow less and finally fail to appear.

at all This appears to be associated with the building of a tolerance because thereafter the dosage may be gradually increased without reactions

III The localizing reaction occurs with less consistency than the above described ones It is a most interesting and helpful phenomenon when dealing with a general disease arising so frequently from closed foci of infection, in themselves so quiescent as to escape clinical detection

This reaction is an acute activation of inflammation in some quiescent focus of infection which comes on with the secondary phase of the general reaction and is noted most often following the first or second minute trial doses used in beginning a course of treatment As mentioned these doses are most apt to excite sharp and stormy general symptoms coming on within twenty four hours after their injection This reaction has most frequently been seen as the lighting up of acute inflammation in nasal accessory sinuses In a few instances acute alveolar abscesses about the apices of devitalized teeth which recent x-ray evidence had not condemned, were most striking instances of this type of reaction

IV Local hyperemia at the site of intradermal injections of antigen is noted occasionally These areas are at their height from eighteen to twenty four hours after an injection They amount to faint pink macules, or very slightly raised flat papules, seldom exceeding 1.5 cm. in diameter and typically surrounded by a narrow zone of blanching which merges with the normal appearance of the surrounding skin These reactions have been noted following the intracutaneous injection of 0.05 cc. of the 1:100 million dilution Patients showing these reactions do not appear to differ in their general hypersensitivity, as judged from the other reactions, from patients who do not show them

This reaction appears to be of little aid clinically and is too inconstant to be of any practical importance

PRESENT RECOMMENDATIONS IN THE USE OF THE ANTIGEN OF STREPTOCOCCUS CARDIOARTHRITIDIS

The consideration of paramount importance in employing Streptococcus cardioarthritidis soluble antigen for therapeutic purposes is that of so regulating the individual doses that no noteworthy reactions follow them This means that best results are to be obtained with very small doses The fact that overdosage delays the onset of reactions must always be borne in mind because with such doses administered at the four day intervals recommended their cumulative effects may amount to nothing more than a general activation of the disease in which the reactions to individual doses are entirely obscured Since the objective and subjective symptoms constituting the general and focal reactions to this antigen are identical with those exhibited in a spontaneously arising exacerbation of the disease, it is only natural when the delayed symptoms from overdosage appear, to regard them as a natural exacerbation of the disease unassociated with the treatment If this is done, however, it practically always means failure in this form of treatment From a practical standpoint, the best results will be obtained for the patient *if all such activations of symptoms are ascribed to overdosage of antigen and further reduction of dosage practiced accordingly* Our experience has been that if reactions of all sorts can be avoided,

the patients do remarkably well. We therefore recommend beginning with 0.05 c.c. of the 1:1000 million dilution injected intradermally. This initial dose depending on the patient's degree of hypersensitiveness, will excite within the first forty-eight hours, rarely, the primary type of reaction, frequently, a period of euphoria, or, quite commonly, the secondary type of reaction. In the first instance, the dose is very much too large, in the second, it is much too large, and in the third, while still too large, it is approaching the proper amount. In the first instance, reduce dosage to one-hundredth of the initial amount, in the second instance, to one-tenth the original dose, and in the latter case, continue the dosage constant upon repeated subcutaneous injections every four days. The secondary type of reaction will come on earlier, be milder and of shorter duration with each repetition until no reaction whatever can be detected. The dosage now may be regarded as properly adjusted for the particular patient. After reactions fail to appear following the injection of the dosage as adjusted, additions of 25 per cent to 50 per cent increments may be made from time to time. If any increased dosage elicits a reaction, this is the signal for repetition of dosage without further increase until a reaction fails to follow the injection.

In this plan it is more important to avoid reactions than to try to build up the dosage. Dosages without perceptible reactions following them appear to build tolerance but excessive dosage appears to hinder all semblance of the building of tolerance. The usual finding is that one will have to repeat the dosage which elicits a prompt general reaction from three to eight times before any additions to it may be practiced with benefit. It thus appears that patients will continue with the 1:1000 million dilution for a month or more before the dosage is built up to such volume that it is more conveniently administered by employing the 1:100 million dilution. This latter being ten times the strength of the former dilution furnishes equivalent doses in one-tenth of the volume attained with the former dilution.

In this manner tolerance may be built up slowly so that in certain individuals with long standing chronic arthritis one may after three months or more, pass to the 1:10 million dilution, or finally to the 1:1 million dilution. These latter dilutions are employed much less frequently in practice than the first two because in the less obstinate cases complete quiescence of the disease may be obtained with 1:1000 million alone, or when it is supplemented by the 1:100 million dilution. The benefits gained may or may not be permanent. Relapses occur frequently, so that two recommendations further are made.

1. In patients with rheumatic fever, antigen is not used except as a follow-up treatment after the antiserum. The course is begun after the period of serum disease has passed, namely, twelve to seventeen days after the initial injection of the antiserum. After carrying the patient up to 0.5 c.c. of 1:100 million dilution the course is discontinued after repeating this dosage three times. The patient is then advised to take each spring and fall, for several years, a short course of antigen just prior to the season of greatest prevalence of rheumatic fever. These courses are begun with 0.05 c.c. of 1:1000 million dilu-

tion and built up to a tolerance of twenty to fifty times this amount. This can usually be done in six to twelve injections.

2 In chronic arthritis after the initial course of active treatment with injections every four days has eliminated the signs of active inflammation in joints, the intervals are prolonged to seven, ten, fourteen, twenty one, and finally to twenty eight days. A dosage of about one tenth to one twentieth of that attained in the more active course of treatment is now administered every month for a year or more. This plan appears to be effective in maintaining the improvement gained and in preventing relapses.

The following selected protocols illustrate certain features of the technique in the therapeutic application of the soluble antigen of *Streptococcus cardioarthritidis* in cases of chronic arthritis.

CASE 3—S S White female aged twenty years typist. Diagnosis atrophic arthritis.

This patient when first seen on March 4 1929, was suffering from a multiple arthritis of the atrophic type affecting particularly the small joints of the hands, the wrists, the right elbow, and right knee. She had diphtheria and measles as a child and also tonsillitis occasionally. At the age of twelve years she had a painful swollen ankle which returned to normal after a few days. At the age of fourteen years she developed a tenosynovitis with puffy swellings on the backs of both hands, which persisted without any particular inconvenience. Three years ago at the age of seventeen years she developed swelling, redness and pain about the middle joints of the fingers and gradually similar involvements of the wrists, elbows, and knees appeared. The tonsils were removed in 1927 or one year after the onset of the arthritis in the small joints of the hands. Five months before coming to the office she went under the care of a very careful internist who placed her in a hospital for extensive diagnostic studies. No definite focus of infection was found. At the time of the examination she was in great discomfort because of multiple swollen, tender and painful joints of which both wrists, the metatarsophalangeal joints, the phalangeal joints and right ankle were the most active. The fingers presented the typical fusiform appearance. The interosseous muscles of the hands were greatly atrophied and the puffy swellings of tenosynovitis appeared on the backs of both hands. She weighed 85½ pounds, was 61 inches tall and showed a regular pulse of 84 per minute. The temperature was 99.2° F and the blood pressure 120 mm mercury, systolic and 68 mm diastolic. Careful examination revealed a subacute pharyngitis, slight impairment as of thickened pleura over the lower right chest posteriorly and a soft systolic murmur at the cardiac apex which was not transmitted outward. The laboratory findings were:

Blood count Hemoglobin 90 per cent
 Erythrocytes 4,880,000
 Leucocytes 10,000

Differential Polymorphonuclears 53 per cent Lymphocytes 43 per cent
 Blood urea nitrogen 13 mg per 100 cc blood
 Blood uric acid 3.8 mg per 100 cc blood

Opsonic index of the blood serum against *Streptococcus cardioarthritidis* was 1.2 and it agglutinated suspensions of *Streptococcus cardioarthritidis* up to dilutions of 1:640.

Immediately prior to coming under treatment from January 13, to February 23 1929 she had received six injections of *Streptococcus cardioarthritidis* soluble antigen. The 1:10 million dilution was employed subcutaneously at weekly intervals in increasing dosages from 0.05 cc to 0.5 cc. The reactions with pain, swelling and redness about the joints were so severe following these treatments that she discontinued them. From her account of the symptoms following the injection of 0.5 cc of 1:10 million dilution of *Streptococcus cardioarthritidis* soluble antigen on February 23 it was evident that she had had both the primary and the secondary phases of the general reaction and consequently her treatment was started.

on March 7 with one one-hundredth of the dosage last received, namely, 0.05 cc of the 1:100 million dilution. Upon studying her reactions to this amount it was still regarded as too large and was reduced to 0.03 cc and later to 0.02 cc. The following represent the amounts used in the first twelve treatments at weekly intervals and serves to illustrate the process of adjusting dosage to the individual case: 0.05, 0.05, 0.03, 0.03, 0.02, 0.02, 0.03, 0.03, 0.05, 0.07, 0.07, 0.1 cc. These treatments covered the period from March 7 to May 24, inclusive. From May 24, to July 12 inclusive, weekly injections of 0.1 cc were used, and thereafter until April 17, 1930 an injection every two weeks was practiced—the amounts used per dose were 0.05 cc to 0.1 cc of the 1:100 million dilution. From April 27, 1930 to date the amount has been continued at 0.1 cc of the 1:100 million dilution per injection but the treatments have been spaced at intervals of three weeks.

No foci were removed in this patient, and no medicine administered. Following the fourth injection in the course, the patient returned to her work as a typist and has continued uninterruptedly since. She has remained free from subjective symptoms and objectively no signs remain about any of the joints, except that from time to time a very slight puffiness from the tenosynovitis is noted over the backs of the hands. This case is presented to illustrate

The aggravation of symptoms under the larger dosage of the antigen, the improvement beginning promptly under much smaller dosage, the schedule of dosage as used early in the course in adjusting it to the patient's tolerance, and the plan of maintaining a small dose at lengthened intervals in treatment over a prolonged period after the arthritis has become quiescent.

CASE 4—J. R. White, male, aged fifty-four, printer. Diagnosis: atrophic arthritis with deformities.

This patient was seen first in October, 1929. He had suffered from a multiple arthritis for the past thirteen years, and presented the ulnar deviation of the fingers of both hands so characteristic of arthritis deformans. His father, two brothers, and a sister had died of pulmonary tuberculosis. The patient had had in childhood, measles, chickenpox, pertussis, and mumps. He could recall no other illness except that he has been particularly susceptible to "colds in the head." For several weeks prior to the onset of the arthritis, which began in the joints of the fingers, he presented an irregular temperature which was diagnosed by his physician as "intermittent fever." He has never suffered greatly from pain in conjunction with the swollen, stiffened joints. The ulnar deviation of the fingers has been noted for seven or eight years. There has been very gradual progress of his joint involvements, so that during the years since the onset he thinks that it has at one time or another involved "practically every joint in the body." He has been able to continue his work as a printer but for the past four or five years has been greatly handicapped because of stiffness and deformity of the hands.

Upon physical examination the outstanding features were the multiple distorted and deformed joints, the undernutrition, the atrophy of the skeletal muscles, and considerable disability because of moderate contractures of the wrists and elbows, and marked limitation in the movements of the fingers, wrists, elbows, shoulders, knees, ankles, and toes. The patient was sixty-eight inches tall and weighed one hundred forty-three pounds. The temperature was 98.6° F, the pulse regular at eighty-one beats per minute, and the blood pressure was 136 mm of mercury, systolic and 84 mm diastolic. The left tympanum showed a perforation but no inflammation. The inferior turbinates were large and the mucous membranes of the nose and pharynx were hyperemic. An excess of clear mucus appeared on the posterior pharyngeal wall. The frontal and maxillary sinuses were clear. The tonsils were small and without gross evidence of infection. Many of the teeth had been extracted. The remaining ones showed no evidence of apical infection upon transillumination nor by x-ray examination. The findings over the pulmonary and cardiac areas and over the gall bladder and appendix did not reveal anything of note. There was a slight distension of the bursae about the knees and considerable of those about the ankles. A pitting edema of both legs extended upward over the lower third of the tibiae.

The laboratory findings were Hemoglobin 80 per cent, erythrocytes 4,140,000 and leucocytes 5,000 per cmm, polymorphonuclears 68 per cent, lymphocytes 26 per cent mononuclears 2 per cent eosinophiles 4 per cent Blood urea nitrogen 11 mg, blood sugar 100 mg, and blood uric acid 3.4 mg per 100 cc blood Throat culture negative for Streptococcus cardioarthritidis Opsonic index for Streptococcus cardioarthritidis was 0.8 Sedimentation of erythrocytes was 16 mm. in one hour Urine clear yellow, acid, specific gravity 1.020, no sugar no albumin no casts, no pus cells

This patient was treated with the antigen of Streptococcus cardioarthritidis (Table III)

TABLE III

							BODY WEIGHT POUNDS
10/18/29	0.1 cc	of 1	1000 million	dilution	intradermally		142 $\frac{1}{2}$
10/25/29	0.2 cc	of 1	1000 million	dilution	subcutaneously		142 $\frac{1}{2}$
11/ 1/29	0.05 cc	of 1	100 million	dilution	subcutaneously		143 $\frac{1}{4}$
11/ 7/29	0.2 cc	of 1	100 million	dilution	subcutaneously		143 $\frac{1}{2}$
11/14/29	0.1 cc	of 1	10 million	dilution	subcutaneously		144
11/21/29	0.06 cc	of 1	1 million	dilution	subcutaneously		144 $\frac{1}{2}$
11/29/29	0.3 cc	of 1	1 million	dilution	subcutaneously		144
12/ 7/29	0.03 cc	of 1	10 thousand	dilution	subcutaneously		144 $\frac{3}{4}$
12/14/29	0.15 cc	of 1	10 thousand	dilution	subcutaneously		144 $\frac{3}{4}$
12/21/29	0.15 cc	of 1	10 thousand	dilution	subcutaneously		143 $\frac{1}{4}$
12/28/29	0.2 cc	of 1	1 million	dilution	subcutaneously		145 $\frac{3}{4}$
1/ 4/30	0.4 cc	of 1	10 million	dilution	subcutaneously		145 $\frac{3}{4}$
1/11/30	0.08 cc	of 1	10 million	dilution	subcutaneously		144 $\frac{3}{4}$
1/18/30	0.15 cc	of 1	10 million	dilution	subcutaneously		146 $\frac{3}{4}$
1/25/30	0.3 cc	of 1	100 million	dilution	subcutaneously		144
2/ 1/30	0.1 cc	of 1	100 million	dilution	intravenously		144 $\frac{1}{2}$
2/15/30	0.1 cc	of 1	100 million	dilution	intravenously		145 $\frac{1}{4}$
3/1 /30	0.2 cc	of 1	1000 million	dilution	intravenously		144 $\frac{1}{4}$
3/15/30	0.2 cc	of 1	1000 million	dilution	intravenously		145 $\frac{3}{4}$
4/ 5/30	0.2 cc	of 1	1000 million	dilution	intravenously		147
4/26/30	0.2 cc	of 1	1000 million	dilution	intravenously		147 $\frac{1}{4}$
5/17/30	0.2 cc	of 1	1000 million	dilution	intravenously		148

This patient exemplifies the low grade indolent type of chronic arthritis which grew worse slowly but very persistently in spite of a great variety of measures used in its treatment

The complete protocol of the injections is presented to show the rapid increase of dosage, in contrast with the case presented above and to emphasize particularly that dosage must in all cases be adjusted to the individual patient In this case moderate focal reactions were elicited with the rapidly increasing doses, but general reactions were not noted even though the dosage was carried up to 0.15 cc of a special dilution of 1 10,000 Thereafter it was gradually reduced in an effort to find that amount which appeared best suited to this case After some weeks a basic amount of 0.2 cc of the 1 1000 million dilution administered every two weeks was established

The improvement in this patient was unmistakably noticeable following the fifth injection, and has continued steadily since He has gained steadily in strength so that he can go through his day's work without experiencing the inordinate fatigue formerly so trouble some The arms which could scarcely be raised to the horizontal position now are readily raised above the head and the patient experiences the great pleasure of buttoning his collar in the back, combing his hair, and scratching the back of his neck The edema of the legs and the distension of the bursa about the ankles have disappeared The patient is bright and cheerful instead of baggared and morose By means of special exercises he is cheerfully cooperating in attempts to overcome the contractures of the wrists and the deformities of the hands Progress in this is being made but much remains yet to be accomplished

SUMMARY

A special technic is necessary in the use of the antiserum of *Streptococcus cardioarthritidis* in the treatment of rheumatic fever in order to avoid the undesirable "focal reaction" following serum administration. This technic is presented in detail.

The possibilities of an aqueous extract of *Streptococcus cardioarthritidis* in the treatment of rheumatic fever, as well as chronic atrophic and hypertrophic arthritis are discussed. In its use the importance of regulating dosage so as to avoid focal and general reactions is emphasized. The present development of the author's technic and its application is recorded.

These products and the methods of their therapeutic application are to be regarded as in the developmental stages, so that further modifications are to be expected.

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N. E. CORNER THIRTY SIXTH AND WALNUT STREETS

THE ALLERGIC JOINT*

BY JOSEPH A. FREIBERG, M.D., AND STANLEY C. DORST, M.D., CINCINNATI

GENERALLY speaking there are two types of arthritis which are identified in various ways. The type of arthritis variously known as hypertrophic, degenerative, or osteoarthritis, is recognized almost universally as a noninfectious, combined osseous and chondral degeneration appearing after the fifth decade of life, accompanying similar changes in all of the other body tissues. The characteristic findings of this type of degenerative arthritis are readily recognized, and the pathologic picture is well understood.

The second type of arthritis which is classified by various writers as atrophic, proliferative, infectious, or rheumatoid arthritis, encompasses a great number of dissimilar joint lesions with only a single characteristic sign, inflammation. Under these headings are found multiple, progressive joint involvement, persistent inflammation localized in a single joint and fleeting joint lesions which heal spontaneously. Obviously, some differentiation must be made on a clinical and etiologic basis between these varied subtypes of this group of arthritides, before a therapeutic régime can be developed.

In this paper we will endeavor to show that in addition to the recognized specific arthritic lesions such as the tuberculous, the gonococcal, and other frankly infectious arthritides, there is another type which may be identified by constant signs, both clinical and pathologic. Not only may this type be recognized, but, further, the therapy is the same in all cases. We realize that the term allergy has been used loosely in many instances in the literature; nevertheless we have found no better descriptive term than the allergic joint for the type of disease which we shall describe.

One of the authors (Freiberg)¹ has noted the development of a characteristic joint lesion in rabbits following the repeated injection of a bacterial filtrate. The joint changes which followed the repeated injections of this bacterial filtrate were localized, altered tissue responses, otherwise identified as a hypersensitivity reaction to the products of bacterial growth. It is this disease process which is termed allergy. Following the repeated intraarticular injection of a bacterial filtrate in the rabbit, a monarticular arthritis developed. This arthritis did not become evident until two or more injections of the filtrate had been made. With each successive injection the localized reaction in the joint became more pronounced. Coincidentally, in the rabbits which had received these bacterial filtrate injections, there developed not only specific agglutinins in the blood, as shown by a high titer serum agglutination reaction against the bacterium used in making the filtrate, but also a skin hypersensitivity to the filtrate when injected intradermally. Other factors possibly concerned in these reactions, such as nonspecific foreign proteins and variations in

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hydrogen-ion concentrations, were controlled by numerous experiments and excluded thereby

The arthritic lesions which were produced by the injection of bacterial extracts showed characteristic changes, both clinically and microscopically. The macroscopic changes consisted in a mild periarticular infiltration, a pronounced intraarticular effusion, and a negligible loss in joint mobility or function. In the instances in which the lesion was of several weeks' duration, there was noted a well-marked atrophy of the muscles controlling the motion of the involved joint. The microscopic changes consisted in an extensive hypertrophy and hyperplasia of the synovial membrane with areas of focal collections of lymphocytic infiltration of the synovial membrane and of the subsynovial tissues. The articular cartilage had numerous small areas of necrosis with the formation of an overlying pannus of connective tissue. When new bone formation occurred, this was found at the site of ligamentous attachments, external to the joint surfaces, and in no manner interfered with joint function. A number of these experimentally produced arthritic lesions were observed over a long period of time following the injections of the bacterial filtrate, and though the periarticular infiltration disappeared entirely within several weeks, after several months had elapsed the synovial membrane hyperplasia and the muscular atrophy still persisted. It was interesting to note that the latter changes existed long after the joint effusions had entirely vanished. In fact, in several instances nine months after the last injection of the antigen, the synovial membrane was appreciably thicker than normal, and the knee joints showed a characteristic relaxation.

Of the many types of human arthritis, excluding the specific arthritides, such as the tuberculous, the gonococcal and the septic forms, differentiation into definite groups is always most difficult if the disease is of many years' standing. When an arthritic lesion is progressive in nature, even though it be confined to a single joint, so many changes occur due to the efforts of nature to accommodate the joint to the irritating lesion, that the lesion itself may be almost completely masked. Extensive deformities caused by prolonged overaction of the stronger muscle-groups resulting in actual dislocations and acute contractures of the soft tissues, not infrequently stimulate the formation of new bone in an attempt to limit the pain. Under these conditions, the etiologic factor may be suggested, not by the clinical picture, but by the bacteriologic reactions. From a clinical point of view this group of arthritides is of less importance, because the damage to joint function already has occurred. Our interest has been centered chiefly on the less advanced cases, those in which one or many joints are involved, but few severe deformities have developed. At the present time we are often unable to conclude from a study of cases of the former type what the clinical manifestations of the disease were at the onset, and whether the various types of therapy instituted previously have altered the characteristics of the clinical picture. It is for this reason that we shall confine the discussion in this paper to a group of cases, all similar in many respects, and all reacting in a like manner to the bacteriologic tests and treatment.

The type of arthritis which is designated as allergic has a definite group of clinical findings. The joint, whether it be a knee or a finger, appears fusiform,

has slight or no periarticular infiltration as determined by palpation, shows moderate limitation in function, is distended with fluid, and has a characteristic boggy feel on palpation. Roentgen films show some soft tissue thickening, moderate irregularity, and narrowing of the joint surfaces, but no extensive newbone formation. The history of the lesions frequently goes back many years, and it is also a frequent finding that at irregular intervals normal joints have become involved and have remained enlarged. It is a surprising fact that in the majority of these cases, although pain is present it is rarely severe, and is not the chief factor in causing the patient to seek treatment. It is the unsightly appearance of the joints, and the limitation of activity which have influenced the individual to seek relief. Aspiration of the joints has been of no diagnostic or therapeutic aid in this group of cases, excepting to exclude the various specific arthritides. The fluid obtained on joint aspiration has in each instance been an effusion, yellow in color, clear or very slightly cloudy, and negative on culture. In a series of approximately one hundred cases of non-specific arthritis, slightly more than 30 per cent have been classified as allergic. This statement is based not only on the clinical and laboratory findings of the initial examinations, but also and more certainly, after a period of extensive therapy. No case has been classified as allergic arthritis unless positive intradermal reactions have accompanied the injection of an autogenous vaccine and improvement or complete cure has followed a period of treatment.

It seems necessary, at this point, to digress and briefly review a series of clinical bacteriologic studies which have been of great importance in determining our treatment of these cases.

During the past five years one of us (D), together with his coworkers, has been engaged in a comprehensive study of vaccine therapy in which particular attention has been paid to the selection of organisms to be used as antigens. We have been especially interested in the possible significance of intradermal tests when employed to indicate specific susceptibility to bacterial antigens. The general problem and method of approach was first described by Wherry² in 1927 and was further elaborated by Dorst and Wherry in 1928.³ Subsequent studies are now in the hands of publishers and will appear in the medical literature at an early date. Several hundred patients have been carefully studied including cases of bacterial asthma, ulcerative and nonulcerative colitis, arthritis, certain skin lesions and angioneurotic edema. These clinical studies tend, for the most part to substantiate Wherry's original hypothesis, and we now believe that both susceptibility and sensitivity to bacterial strains may be demonstrated by intradermal reactions. Antigens so selected and given, by the desensitization method, yield excellent therapeutic results. Constantly, throughout our experimental work, two facts have been emphasized, and we have come to regard them as of paramount importance in effective vaccine therapy. The first is the specificity of the antigen and the second the desensitization method of administration. The chaotic state which obtains to day in the field of vaccine therapy is, we believe primarily due to a failure to recognize the importance of these factors. Stock vaccines have failed because they completely disregard the factor of specificity. Autogenous vaccines have likewise often failed, first, because there seemed to be no method of determining

specificity, and secondly, because the specific organism selected by chance was usually given in massive doses similar to those used to protect noninfected individuals. Vaccine so given often resulted in the exaggeration of symptoms. These facts have contributed to the low status of vaccine therapy.

It seems advisable to point out a third fact which we have had brought home to us repeatedly, namely, that immunization of a noninfected individual against a specific organism is one problem, and the desensitization of an already sensitized individual is quite another, and cannot be accomplished by using massive doses. We may not go further into the discussion in this paper, the experimental studies on this subject are available for review, but we will state our general contention as follows. Individual susceptibility to a given bacterial species may be determined by intradermal reactions to properly prepared antigens. This fact is of great usefulness when one is working with an extremely varied flora.

It seemed essential to give the preceding survey as a background to a presentation of our clinical experiments with certain types of arthritis. While we are not ready to state that a positive intradermal test always indicates an allergic reaction between the host and the "active" antigens, this seems in many instances to be the case. We are certain that in our cases of asthma, of angioneurotic edema, and in many of our nonulcerative colitis cases we are dealing with patients whose disease is of an allergic nature, if we may accept the more comprehensive meaning of the term allergy, and that the positive intradermal reactions which we obtain are local manifestations of the allergic state. We have presented experimental evidence suggesting that a certain type of joint lesion is probably allergic in nature, and it now remains for us to show how this concept has been verified by clinical experience.

As suggested above, the group of arthritides with which we have been working have previously defied accurate classification. In certain respects they suggest infectious lesions: there is swelling, local heat, and subsequent atrophy of adjacent muscle groups, but here the similarity to known infectious arthritis of the "specific" type ceases.

If the fluid removed from such joints be carefully studied, we note many points of difference when compared with fluid from known inflammatory lesions. This fluid is thin and straw-colored, the cells present being chiefly lymphocytes or large mononuclears, and the total cell count is low. The general characteristics of such fluid suggest a transudate rather than an exudate. We have studied many such specimens bacteriologically, exposing the material to enriched media under various tensions of O and CO₂, but have not succeeded in isolating organisms. The time element has been extended to twelve days and then to fifteen days, and still our cultures have remained sterile. It has been suggested that these lesions may be due to some unusual form of streptococcus. We have failed repeatedly to recover it, but granting that this failure may be due to lack of technical skill, we are still at a loss to understand a pyogenic infection whose characteristics are so unlike any other recognized as such.

In our opinion such lesions are not due to active and maintained infections in the joints themselves, but are rather the expression of a sensitized tissue to

an antigen which remains in a persisting focus elsewhere in the body. Such a sensitization may be brought about in two ways: either from repeated exposure to soluble toxic products from a distant focus, or from transient bacteremias, during which the joint cavities have been "seeded" with organisms which have failed to grow, due perhaps to reduced oxygen tension. Experimental sensitization may be produced in either way, but we are inclined to favor the concept of circulating sensitizing toxins. A joint so sensitized may be expected to "flare up" whenever it is reexposed to the sensitizing antigen, and in many instances a focus far removed from the involved joint may be a constant source of supply for the antigen which keeps the allergic reaction "active." When such distant foci are removed surgically we frequently accomplish a striking cure, but more often the removal of foci is either impossible or incomplete, and the patient's symptoms recur. It is with such cases that we have been chiefly concerned.

Each case is studied carefully from the standpoint of foci of infection. Cultures are made from postnasal secretions from material obtained from sinus puncture, from suction in the ethmoid region from infected teeth upon removal, from tonsils and from the contents of the enteric tract. If an obvious focus presents itself it is given careful attention, but in the majority of cases we must attempt an exhaustive study of the flora of the mucous membrane lined tracts of the body for these form the great avenues of entrance. All the organisms obtained on various types of media and under differing gas tensions³ are isolated in pure culture and skin tests are made with each individual strain. Sensitivity is indicated by marked redness, induration and local heat. These reactions reach their height from twenty-four to thirty-six hours after the injection and disappear slowly. Organisms giving rise to such reactions are designated as "active strains" and are used singly or mixed as the case may be for the purpose of desensitizing the patient. It may be stated that organisms giving reactions in these cases of arthritis come most frequently from the upper respiratory tract and the colon. Often they are organisms usually designated as "normal flora," and a Freidlander's bacillus from an antrum or a strain of *B. coli* from the colon will when injected often give a marked skin reaction and a simultaneous focal reaction in the involved joint with an acute exacerbation of the clinical picture. This focal reaction gives added weight to our opinion concerning the allergic nature of the disease.

In desensitizing our patients the active strains are employed in a dilute suspension, and very small amounts of the antigen are given at each injection. Usually we begin with 0.5 minim, carefully measured from a tuberculin syringe. This dosage is continued for several days and then increased to 1.0 minim. Injections are given every day or every second day and the dose is slowly increased by 0.5 minim intervals. Often, in dealing with extremely sensitive individuals it is necessary to continue at the 1.0 or 1.5 minim dose for days before it can be increased. We try to avoid any marked local reaction and all focal or general reactions for we have learned to our sorrow that haste usually results in a severe focal "flare up," and it is then necessary to start again from the beginning.

CLINICAL STUDIES

CASE 1—Mrs H C, thirty three years of age, married Onset sixteen years ago with swelling and pain in both knees After nine months the right knee became well, and left knee improved Excepting for a period of hospitalization in 1925, she has been up and about the entire time She has noted crepitation in the left knee at all times In May, 1928, the left knee became very swollen and painful At irregular intervals she has had slight discomfort and stiffness of the fingers Otherwise the history is negative excepting for constipation with occasional intermittent attacks of diarrhea, during which there would be traces of mucus and blood in the stool

The first examination on October 3, 1928, showed a tall slender type of individual, weighing 123 pounds The left knee was distended with fluid and motion was limited to an arc of 60° to 160° There was marked crepitus in both knees noted on motion The other joints showed no abnormal changes X ray films of the knees were negative General examination revealed no static abnormalities in the feet or legs, but there was a slight fatigue posture Aspiration of the left knee on October 10, 1928, yielded 150 cc of a rather turbid serous fluid with further amount of fluid remaining in the joint This was sterile on culture

Prior to her first visit, repeated attempts had been made in several clinics to find and remove some focus of infection The teeth, sinuses, and throat had been carefully studied without positive findings The one rather definite lead came in the gastroenteric history Nevertheless cultures were made from materials obtained from postnasal pharynx, and after suction applied to the ethmoid region Cultures were also made from the stools, and these alone gave significant findings Four strains of organisms were isolated from the nose and throat secretions, and six from the stools These were recovered in pure culture, harvested, and heat killed at 60° C Intradermal tests were carried out using all nine strains, and strongly positive reactions were given by *B coli communior* and *B mucosus capsulatus* These two strains were incorporated in a mixed vaccine, and treatment was started, using 0.5 minims of a dilute suspension The vaccine was then sent to the patient's family physician with instructions regarding its administration He, however, failed to recognize the importance of advancing the dosage very slowly, and in a period of fifteen days increased the amount from 1 to 8 minims The result was an acute exacerbation of symptoms with effusion not only into the left knee, but also into the right knee, and when examined at this period, her symptoms were definitely more marked than when first seen

The dosage of vaccine was reduced to 0.5 minims and then given in very small doses so that between December 20, 1928, and February 23, 1929, it had been increased only to 2 minims When examined at this time, she showed little improvement The following brief summary covers her progress during the next eighteen months Throughout this time she carried on her usual routine and was at no period subjected to enforced rest

March 21, 1929, definite improvement Dosage 2 minims

April 16, 1929, improvement continues, fluid markedly decreased in amount Dosage held at 2 minims

May 6, 1929, no pain, only slight effusion in left knee, right knee well Vaccine dosage 2.5 minims

June 10, 1929, improvement continues States that she is better than she has been for many months

October 8, 1929, no pain, practically no fluid

January 3, 1930, new vaccine prepared from colon flora still slightly sensitive to *M capsulatus* and definitely sensitive to *B coli* The new vaccine was somewhat more concentrated than the first with the result that there was a marked focal reaction with return of fluid to the left knee following the third 2 minim dose The vaccine was diluted and the dose reduced to 1 minim

February 4, 1930, feeling well No fluid in either knee

March 28, 1930, no subjective symptoms, no fluid Vaccine 2 minims

June 15, 1930, improvement holds, patient is taking long walks and plays golf with out unfavorable results

CASE 2—Mr A F This patient gave a history of fleeting joint symptoms during the past fifteen years. More recently he has had at least three severe attacks annually, involving feet, ankles, and occasionally the elbows and knees. Each attack has been followed by less complete recovery. Preceding each illness, the patient had some generalized disturbance accompanied by malaise. Excepting for an acute gastroenteric disturbance while in the Philippines in August, 1928, the patient could recall no illnesses. No probable foci of infection had been discovered. In January, 1929, he had acutely painful and swollen feet and swollen right knee necessitating bed rest. Under another physician's care he was placed on a purin free diet for three months, with no relief.

Examination on April 18, 1929, showed effusion in the left knee joint, swollen and acutely tender feet, and ankles associated with marked muscular spasm in the feet and congenital pronation of the feet. X ray films of the knees were entirely negative. X ray films of the feet and ankles showed some narrowing of all of the tarsal and metatarsal joint spaces with decrease in normal density of the bones. Though conscious of no upper respiratory disturbance and in spite of the absence of tonsils, the patient had an injected granular pharynx. Cultures were taken from the postnasopharynx and the stools were cultured. Antigens were prepared as outlined in Case 1. He was not sensitive to any of the colon flora, but gave marked reactions to a nonhemolytic streptococcus from the throat and to a strain of *B. Friedlander* from the same source. Vaccine was prepared in the manner described above. On May 3, 1929, the left knee was greatly improved, though the feet showed no improvement. Iodides and cinchopen had been administered since April 18. Vaccine therapy was started on May 10, the patient still was confined to bed. On May 22 he was permitted limited activity with support for pronation of the feet. Improvement continued with resumption of normal activities until November 6, 1929. No dietary restrictions had been imposed. On this date the patient developed an acute upper respiratory infection with temperature of 101.6 F associated with effusion into the left elbow and severe pain in the feet. This followed a period of two weeks without vaccine therapy. On November 14, having recovered from the acute upper respiratory infection, he was again skin tested with the vaccine, and developed a 4 cm reaction. Vaccine therapy was resumed. Two weeks later pain had entirely subsided and the patient engaged in normal activities. Vaccine therapy was continued for three months. Following the disappearance of inflammatory reaction about the feet and ankles, the patient was given physiotherapy to restore joint mobility. At the present time July, 1930 the patient states that he is free from pain and has engaged in all activities associated with those of an active business man since November, 1929. At no time during the preceding five years has he felt as well, nor has he had such a long period of freedom from arthritic symptoms.

The two cases outlined above serve to illustrate our method of procedure. The scope of this preliminary report does not permit a detailed presentation of a larger series of cases, but Table I gives interesting data obtained from a group of thirteen. Each of the patients included in this table has been as carefully studied as the ones described. No patient is included who has not been under treatment for more than nine months, and the average time since treatment was started is eighteen months.

As an adjunct to the vaccine therapy outlined, careful attention has been paid to any existing deformities and postural abnormalities. During the active phase of the disease nonoperative correction of deformities has been carried out by means of mechanical support and physiotherapy. Muscular atrophy and postural defects, when present, have been treated by carefully supervised exercises and the application of one of the various forms of external heat. In certain instances a period of bed rest seemed indicated. Surgical correction of deformities, when necessary, was not attempted until all evidence of activity in the lesion had disappeared.

TABLE I

CASE	DURATION	PARTS INVOLVED	FOCUS	ORGANISMS SENSITIVE	RESULT
1	3 years	Right knee and elbow	Sinus	Streptococcus hemolytic	Cured
2	18 months	Knee and sacro iliac	Sinus	Staphylococcus hemolytic	Cured
3	14 years	Both knees and spine	Sinus	Streptococcus nonhemolytic	Cured
4	9 years	Both knees	Colon	Bacillus coli, Bacillus mucosus capsulatus	Cured
5	2 years	Spine and elbow	Antrum	Bacillus coli, Streptococcus hemolyticus	No improvement
6	2 years	Both knees and ankles	Throat	Streptococcus, green coloration	Cured
7	4 years	Both ankles	Ethmoid	Streptococcus, nonhemolytic Bacillus of Friedlander	Cured
8	6 months (flu)	Right knee and right elbow	Ethmoid	Streptococcus, green coloration	Cured
9	3 years	Right knee	Colon	Streptococcus, hemolytic Bacillus coli	Marked improvement
10	6 years	Spine and both hips and shoulders	Colon	Bacillus coli, hemolytic (only)	Marked improvement
11	4 years	Knees	Colon	B. coli hemolytic	No improvement
12	5 years	Wrist, elbows and knees	Antrum and ethmoid	Staphylococcus hemolytic Green producing streptococcus	Marked improvement
13	2 years	Right sacroiliac	Tonsils	Hemolytic streptococcus	Improvement

SUMMARY

Our conclusions may be outlined as follows

1 We believe that we have demonstrated the experimental production of an allergic lesion in the joints of rabbits

2 In cases of joint disease in the human being, we encountered a lesion similar to those produced in rabbits. We are not satisfied that any previous explanation of this type of joint lesion in human beings is adequate

3 We believe that this lesion in the human being is also allergic, and that we have demonstrated the relationship between antigenic substances from distant foci and the diseased joint

4 The success of our treatment, which consists, essentially, in desensitizing patients to autogenous antigens which give positive reactions, seems to justify this conception

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A MICROPHOTOGRAPHIC STUDY OF FIVE VARIETIES OF ARTHRITIC STREPTOCOCCI

By DAVID THOMSON,* O B L, M B CH B (EDIN) D P H (CAMB) AND
ROBERT THOMSON,† M B, CH B (EDIN) LONDON

SEVERAL varieties of streptococci have now been incriminated as causes of chronic arthritis. Extensive researches have been carried out on this subject by Dr Warren Crowe in England and by Drs Burbank and Hadjopoulos in America.

These arthritic or "osteotropic" streptococci have been isolated from the blood, urine, feces and apices of bad teeth of patients suffering from chronic rheumatism. When injected into animals such as rabbits it has been found that they produce swelling of the joints and lameness and they can be recovered from the bones and joints of these animals.

Warren Crowe (1928) contributed an important paper on this subject in the *Annals of the Pickett Thomson Research Laboratory* 4: 398-408, with ten illustrative plates.

The several varieties of these "osteotropic" streptococci can be clearly differentiated by their colony appearance when cultivated on Crowe's medium for three days.

Warren Crowe has discovered at least four varieties of "osteotropic" streptococci. These are (1) *Streptococcus zymogenes* (Braxton Hicks) synonymous with *Streptococcus griseus* (American writers) see Plate V, (2) *Streptococcus B 7* (2) h (Crowe's classification) see Plate I, (3) *Streptococcus B 9* (1) a (Crowe's classification) see Plate II, (4) *Streptococcus B 9* (3) a (Crowe's classification) see Plate III.

In April 1929, Dr Burbank sent five strains of his arthritic streptococci to Dr Warren Crowe for investigation. In his explanatory letter he states that "One is of blood culture origin, from the human being; one isolated from the stool, and joints experimentally produced in rabbits with it, three were originally from human blood cultures, and afterwards isolated from rabbits after having experimentally produced low grade joint involvement of long duration."

On investigating these five strains Warren Crowe gave the following opinion as to their identification:

Burbank strain	(1)	<i>Streptococcus zymogenes</i>	(D 3 (1) Crowe's classification)
"	"	(2) <i>Streptococcus fecalis</i>	(D 1 (1) a
"	"	(3) Viridans type	(B 9 (1) e ?
"	"	(4) Colorless group	(A 1 (1) b
"	"	(5) <i>Streptococcus zymogenes</i>	(D 3 (1) " "

After this preliminary identification, Dr Warren Crowe sent these Burbank strains to us for further photographic identification.

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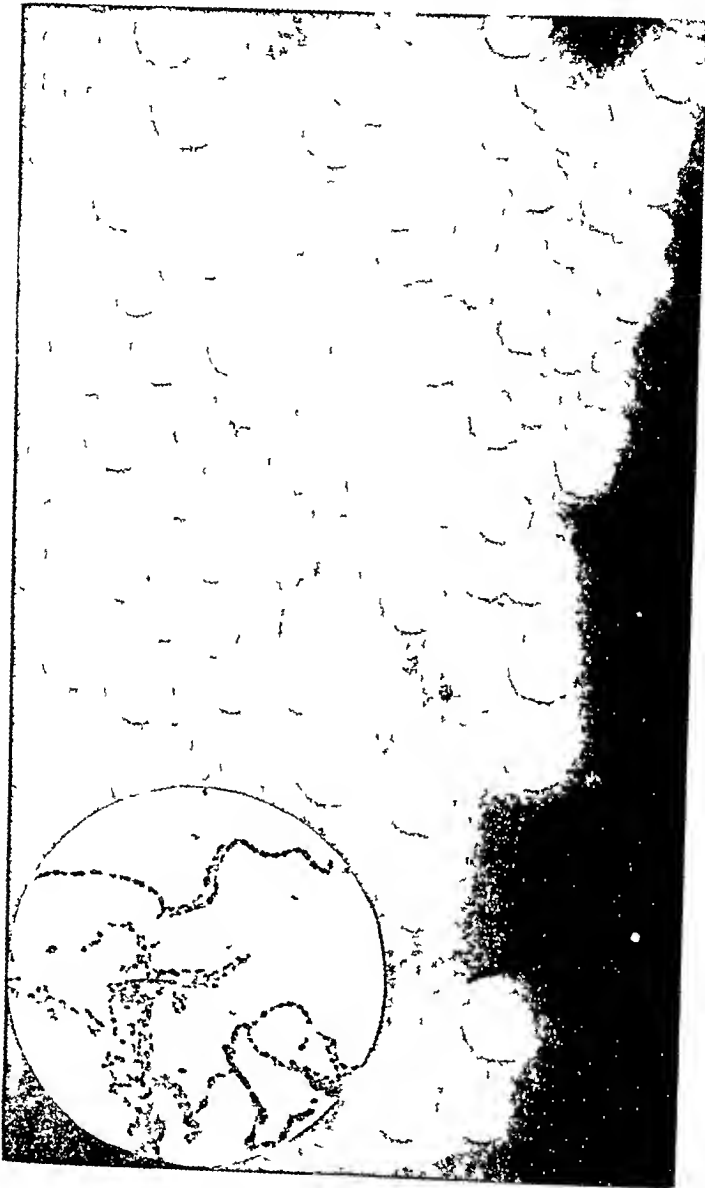


PLATE I

Burbanks Arthritic Streptococcus No III (originally isolated by blood culture)

Microphotograph of the colonies on Crowe's chocolate blood agar after three days aerobic incubation at 37.5 C

Magnified 40 diameters

The colonies are shaped like a cottage loaf. They are soft, moist and glistening with a greenish-yellow halo around them.

Iodine—(Colonies not discolored by Lugol's solution)

Inset Circle—Represents a smear of a broth culture magnified 1700 diameters

SUGAR REACTIONS

Glucose	Maltose	Galactose	Saccharose	Lactose	Mannite	Inulin	Salicin	Raffinose	Litmus Milk
+	+	+	+	+	-	-	+	+	+

Heat Resistance—Negative

This streptococcus resembles very closely Warren Crowe's 'osteotropic streptococcus B 7 (2) h except that the latter does not ferment salicin and does not form such long chains in broth culture.

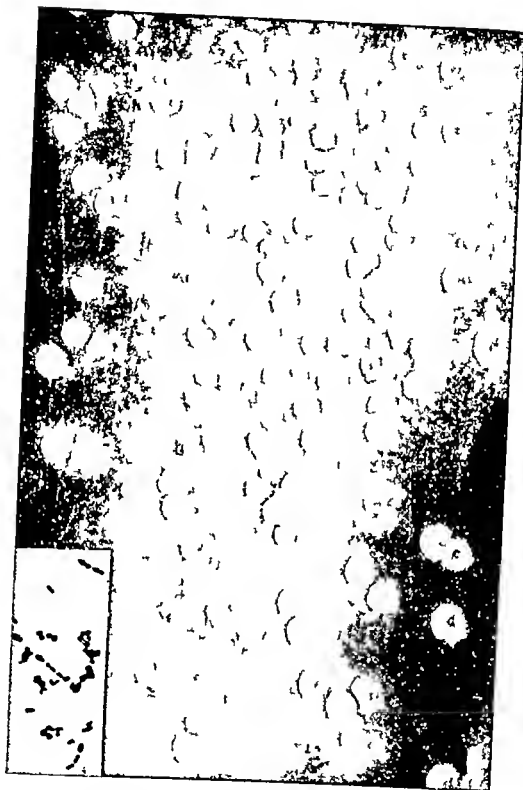


PLATE II

Warren Crowe's Arthritic Streptococcus B9 (1) a.

Microphotograph of the colonies on Crowe's chocolate blood agar after three days aerobic incubation at 37.5 C

Magnified 20 diameters

This streptococcus was isolated from the apex of a tooth from a case of osteoarthritis. The colonies are dry and raised and the majority of them have a central dimple. There is a narrow zone of greenish yellow bleaching of the medium around the colonies scarcely shown in the microphotograph.

Iodine ± (That is the colonies are slightly browned by Lugol's solution more especially the older colonies)

Inset—Is a microphotograph of a Gram stained smear of this organism X1500

SUGAR REACTIONS

Glucose	Maltose	Galactose	Saccharose	Lactose	Mannite	Inulin	Salicin	Raffinose	Litmus Milk
+	+	+	+	+	-	+	+	+	+ Clot
									In hours

Heat Resistance—Negative



PLATE III

Warren Crowe's Arthritic Streptococcus B9 (3)a

Microphotograph of the colonies on Crowe's chocolate blood agar after three days aerobic incubation at 37.5°C

Magnified 25 diameters

This streptococcus was isolated from the apex of a tooth and when injected into rabbits it infected not only the joints but produced hemorrhages in the tendinous portions of certain muscles such as the biceps. It was cultured from the heart blood of the rabbit, also from the joint capsules from the fluid of the joints from the bone of the tibia and from the biceps.

The colonies are soft moist and glistening with a flattish or concave top. They are surrounded by a yellow zone of bleaching not very distinctly shown on the photograph.

Iodine ± (That is the colonies are slightly browned by Lugol's solution)

SUGAR REACTIONS

Glucose Maltose Galactose Saccharose Lactose Mannite Dextrin Inulin Salicin Raffinose Litmus
+ + + + +
+ Inset Circle — Smear of broth culture X1700 — The broth culture shows a copious deposit with a clear supernatant fluid



PLATE IV

Burbank's Arthritic Streptococcus Yo IV

Microphotograph of the colonies on Crowe's chocolate blood agar after three days aerobic incubation at 37.5 C.

Magnified 40 diameters

The colonies are convex soft moist and glistening There is no discoloration of the medium around or under the colonies

Iodine ++ (That is the colonies take on a brown color with Lugol's iodine solution)

Inset Circle—Represents a smear of broth culture magnified 1700 diameters

SUGAR REACTIONS

Glucose	Maltose	Galactose	Saccharose	Lactose	Mannite	Dextrin	Inulin	Salicin	Raffinose
+	+	+	+	-	-	-	-	-	+

Litmus Milk

Heat Resistance—Negative

The colonies resemble those of *Streptococcus fecalis* but it differs from the latter in being nonresistant to heat. The cocci are larger than those of *S. fecalis* and moreover inulin is not fermented by *S. fecalis*.

RECENT RESEARCHES ON THE STREPTOCOCCAL ETIOLOGY OF RHEUMATIC FEVER

BY DAVID THOMSON,* O B E, M B, CH B (EDIN), D P H (CAMB), AND
N GRAY HILL,† M C M B, B S (LONDON), D P H (ENGLAND), LONDON

I PREFATORY NOTE

IN MONOGRAPH I, Vol IV, of the *Annals of the Pickett-Thomson Research Laboratory* (1928) a complete historical account was given by Drs D and R Thomson of the researches which had been carried out on the etiology of rheumatic fever up to the middle of the year 1928. At that time it appeared hopeful that the streptococcus responsible for the disease had been discovered by Birkhaug and Small in America. Another group of workers in America propounded the hypothesis that the disease was simply a state of bacterial allergy to several varieties of streptococci.

During the past two years several workers have attempted to confirm the work of Small and Birkhaug, and we have also carried out extensive investigations on the streptococcal etiology of this disease.

Lazarus-Bailow (1928) Hitchcock (1928), Nye and Seegal (1929), Lazarus-Bailow (1929) and Gray Hill (1929) were all unable to confirm the work of Small and Birkhaug since they found that the *Streptococcus cardioarthritidis* of Small which is identical with Birkhaug's nonmethemoglobin-forming streptococcus was present as frequently in normal persons as in rheumatic fever cases.

Nabarro and MacDonald (1929) investigated the bacterial flora of the tonsils of children with articular rheumatism but were unable to find that this flora was any different from that of nonrheumatic children. Other workers, however, namely Belk, Jodzis and Fendrick (1928), Flinn and others (1928), and Hart (1929) tend to confirm the work of Birkhaug and Small.

With regard to the allergy theory of rheumatic fever several confirmatory researches have appeared by Derick Hitchcock and Swift (1929), Hart (1929) Birkhaug (1929), and Swift (1929).

II GREEN-PRODUCING (VIRIDANS) STREPTOCOCCI IN RHEUMATIC FEVER

Leichtentritt (1929) was able to demonstrate *Streptococcus viridans* in the nodules in two out of seven cases of nodose rheumatism. In one of the cases the same organism was demonstrated simultaneously in the blood.

Cecil, Nicholls and Stamsby (1929) during the spring of 1928 subjected 29 patients with acute rheumatic fever to blood cultures, of whom 9, or 31 per cent, yielded a streptococcus. During the spring of 1929, 31 patients with acute rheumatic fever were studied by blood cultures, of whom 26, or 83.9 per

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cent, yielded a streptococcus. The higher percentage of positive cultures in the 1929 series appears to have been due to improved cultural methods. Of the 35 strains of streptococci recovered from blood cultures 33 have been classified as alpha streptococci (*Streptococcus viridans*), one as a beta streptococcus (*S. hemolyticus*), and one a gamma streptococcus (*S. anhemolyticus*). Some of the *S. viridans* strains produced little green on blood mediums.

Agglutination and absorption tests indicate that the strains of *S. viridans* recovered from the blood of patients with rheumatic fever show a tendency to fall into specific biologic groups. In seven patients with rheumatic fever who were subjected to cultures from affected joints 5 or 71.4 per cent yielded *S. viridans*. In three patients in whom green streptococci were recovered from both the blood and joint agglutination and absorption tests proved the identity of the strains isolated from the two sources. These observations corroborate those of previous investigators and make it difficult to escape the conclusion that rheumatic fever is a streptococcal infection usually of the alpha or *S. viridans*, type. The pathogenesis of rheumatic fever in respect to the joint lesions appears to be analogous to that of infectious arthritis and gonococcal arthritis. Bacterial allergy probably influences the clinical picture in all three conditions, but in each instance the joint manifestations are primarily dependent on localization of bacteria in the joint with subsequent infection.

III OTHER RESEARCHES

Gross, Loewe and Eliasoph (1929) attempted to reproduce in animals the lesions characteristic of rheumatic fever in the human being. A large number of animals representing seven species were employed. Among other materials, streptococci isolated in pure culture from the blood of rheumatic patients (proved to be so by biopsy or by autopsy), as well as the whole blood plasma, serum, pericardial, pleural and hydrocele fluid filtrates from tonsils, subcutaneous nodules, lymph nodes and nasopharyngeal washings obtained from such patients were used in a variety of combinations and with a number of procedures calculated to predispose the animal to the disease. They failed to reproduce the disease.

IV RESEARCHES BY THE AUTHORS

Having failed to confirm the work of Small and Birkhaug, that rheumatic fever was caused by the noncolor producing streptococcus described by them, the authors decided to carry out further researches to ascertain if any other variety of streptococcus was persistently present in rheumatic fever children while being absent from control cases.

This investigation was carried out by a new method devised by one of us (D. Thomson) which consists in compiling extensive photographic records of cultures on Crowe's medium. This method of compiling photographic records had already been applied by D. and R. Thomson to other diseases such as influenza, scarlet fever, measles, tonsillitis, etc. and large numbers of photographs had also been accumulated of the usual flora of normal throats (see *Annals of the Pickett Thomson Research Laboratory* 5, 1929 and future volumes). It was hoped therefore, that if we obtained extensive photographic



PLATE I

Streptococcus Cardioarthritidis — (Small Strain R2)

Microphotograph of colonies on Crowes chocolate blood agar after three days aerobic incubation at 37.5 C. Magnified 40 diameters

The colonies are greyish with a slightly greenish tinge soft moist and cause no discoloration of the medium around or under the colonies. The daughter colonies around the margin may not develop until incubated for several days

Iodine — (That is the colonies are not discolored by Lugol's solution)

Broth Culture — Moderate deposit with turbid supernatant fluid

Inset Circle — Smear of a broth culture magnified 1700 diameters

SUGAR REACTIONS

Glucose	Maltose	Galactose	Saccharose	Lactose	Mannite
+	+	+	+	+	-
Dextrin	Inulin	Salicin	Raffinose	Litmus Milk	
-	+	-	-	+ Clot	

Heat Resistance — Negative

NOTE — This variety of streptococcus is believed by Small to be the cause of rheumatic fever. Small sent three of his strains R1, R2 and R9 to D. Thomson and it was found that they were closely allied streptococci. Strain R1 was Iodine +. Strains R9 and R2 were Iodine -.

R2 was isolated from the faeces. R9 and R1 were isolated from the blood of cases of rheumatic fever. Strain R1 did not ferment lactose and did not produce acid in litmus milk. Compare Birkhaug's strain Plate II.



PLATE II

Vonmethemoglobin forming Streptococcus — (Birkhaug Strain R. F. 36)

Isolated from the tonsil of a case of rheumatic fever

Microphotographs of colonies on Crowe's chocolate blood agar after three days aerobic incubation at 37.5 C Magnified 8 diameters.

The colonies are muddy grey in color with a tinge of green soft moist glistening and very slightly adherent There is no discoloration of the medium around or under the colonies Iodine ++ (That is the colonies are rendered dark brown in color with Lugol's solution)

Broth Culture — Copious white deposit supernatant fairly clear

Inset Circle — Smear of broth culture magnified 1700 diameters

SUGAR REACTIONS

Glucose	Maltose	Galactose	Saccharose	Lactose	Mannite
+	+	+	+	-	-
Dextrin	Inulin	Saline	Raffinose	Litmus Milk	
-	+	+	+	and Clot	

Heat Resistance — Negative

NOTE.—This variety of streptococcus was at first believed by Birkhaug to be the cause of rheumatic fever Several strains have been examined by D Thomson and they have been found to be closely similar to the strains of *Streptococcus Cardioarthritidis* isolated by Small (see Plate I)

Birkhaug found that these streptococci produced a potent toxin which gave positive skin reactions in a large percentage of rheumatic fever cases Birkhaug is now more inclined to believe in the allergic theory of the causation of rheumatic fever

Further microphotographs of these streptococci of Small and Birkhaug will be found in the *Annals of the Pickett Thomson Research Laboratory* 4 19 8 Plates 1 6

records of cultures from the throats of rheumatic fever children, that some difference might be revealed when these were compared with similar photographs already compiled from the throats of normal cases and from various other diseases. Although the majority of the work has been confined to the bacteriologic investigation of the throat in rheumatic fever children, other material was examined when available. Thus blood cultures and cultures from postmortem material, such as the heart valves, pericardium, etc., have been investigated. The most interesting evidence which we possess, however, is that gained by the careful study of cultures from the throats of acute typical cases of rheumatic fever.

a Material Investigated and Technic—One of us (Dr Gray Hill) having charge of one hundred beds for rheumatic fever cases, both acute and convalescent, had ample material for investigation. The culture medium consisting chiefly of plates of Crowe's chocolate blood agar was supplied by the Pickett-Thomson Research Laboratory.

1 **Blood Cultures** From 10 to 20 cc of blood were drawn from the median basilic veins of eight cases of rheumatic fever. The technic employed was that advocated by Cecil, Nicholls and Stainsby (1928). The blood was allowed to clot and was put in the ice box overnight. The tubes were then centrifuged and the serum drawn off with a sterile pipette, care being taken to remove all the serum. The clot was then broken up and transferred to a bottle containing about 100 cc of testicular infusion broth.

In the majority of instances the blood cultures remained sterile. In a few cases staphylococci appeared, but in no instances was a streptococcus cultivated during life.

2 **Throat Swab Cultures** Ordinary throat swabs were taken from over 34 cases of rheumatic fever children. In the majority of instances the tonsils were present and the swabs were taken from their surface. In a few instances the tonsils had been previously enucleated in which case the cultures were taken from the tonsillar fossae. Plates of Crowe's chocolate blood agar were inoculated from all tonsil swabs, and the material was rubbed well into the surface of the medium by means of a polished sterile glass shank. These plates were incubated on the average for a period of three days, and were then photographed by reflected light according to the technic developed by one of us (D Thomson). After several photographs had been taken, these were compared with similar photographs of cultures of throat swabs from normal persons and from other types of disease such as influenza, measles, scarlet fever, ordinary tonsillitis, etc. By the careful study of these photographs it seemed to us that an unusual type of streptococcus with characteristic colonies was constantly present in the cultures from rheumatic fever throats, and this type of streptococcal colony was only occasionally present in the cultures taken from normal throats and from the throats of cases suffering from other diseases. In many instances, more especially in very acute typical cases of rheumatic fever, at least 90 per cent of the total colonies on the culture plate consisted of the unusual streptococcus. In other cases they were present only in moderate numbers but in no case did we find that they were entirely absent. They also persisted in the throat for a considerable time during the period of convalescence from the acute rheumatic fever symptoms.

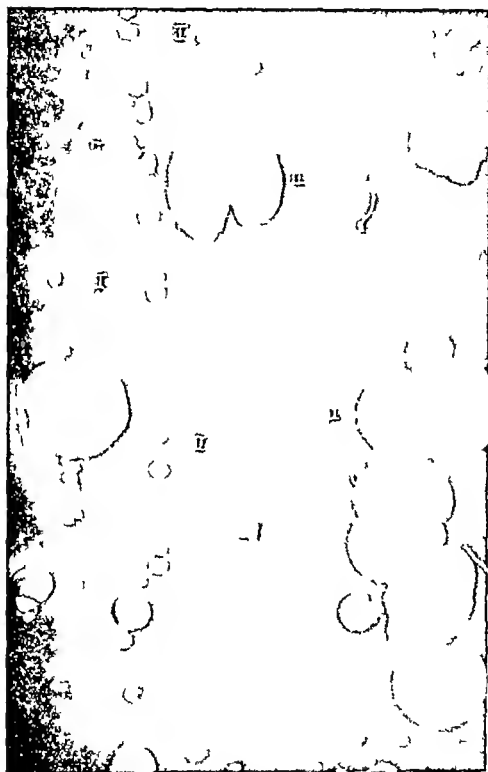


PLATE III

Primary Culture from Rheumatic Fever Throat—(Case Missen. This case had severe chorea.)

Microphotograph of culture on Crowe's chocolate blood agar after three days aerobic cultivation at 37.5 C. (Magnified 20 diameters.)

The predominant colonies (I) are those of a viridans type of streptococcus which has been found by us to be present in large numbers in practically all rheumatic fever throat cultures. In very severe cases these colonies are present in very large numbers (see Plates IV and V). This type of streptococcus appears on the other hand to be more or less absent from the cultures made in a similar manner from normal throats and from the throats of other diseases (see Plates VII to X).

The large colonies (II) are a species of *Staphylococcus albus*.



PLATE IV

Microphotograph of Primary Culture from Rheumatic Fever Throat Swab

Three days aerobic incubation on Crowes chocolate blood agar at 37.5 C (Magnified 30 diameters)

The majority of the colonies (I) are glistening heaped up lens-shaped and surrounded by a yellow ring of bleaching

The colonies are Iodine + or +-

Broth Culture—Shows a clear supernatant fluid with a deposit of flocculent lumps difficult to break up

SUGAR REACTIONS

Glucose	Maltose	Galactose	Saccharose	Lactose	Mannite
+	+	+	+	+	-
Dextrin	Inulin	Salicin	Raffinose		
±	-	-	±		

NOTE.—This was an extremely severe case of rheumatic fever. Cultures from throat swabs taken at weekly intervals always showed large numbers of the heaped-up glistening colonies shown on this plate. These continued to be present for about two weeks after the acute symptoms had subsided after which time the flora became more mixed like that of a normal throat swab.

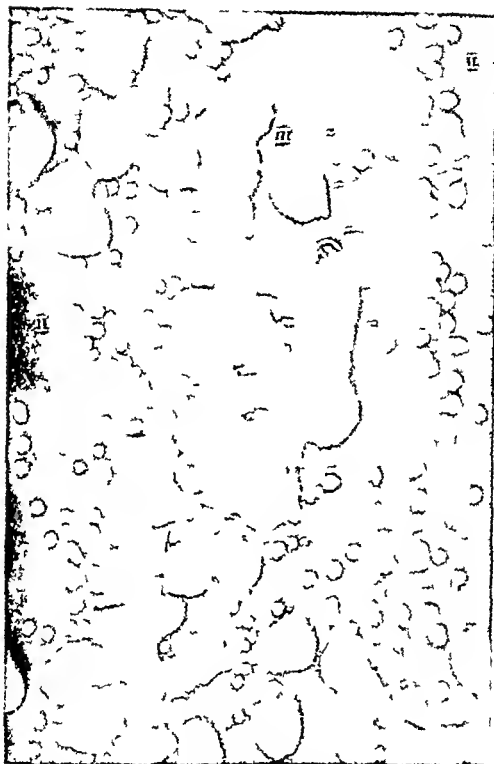


PLATE V

Microphotograph of a Pathogen-Selective Culture from Throat of Rheumatic Fever — (Case Campbell) Three days aerobic cultivation of Crowe's chocolate blood agar at 37.5 C Magnified 25 diameters

NOTE — The pathogen selective culturing in the patient's own blood has killed off all the throat organisms except two namely the *Streptococcus viridans* with glistening lens shaped colonies (I) and a *Staphylococcus aureus* (II)

Colonies (I) are Iodine +

Colonies (II) are Iodine -

The colonies of the rheumatic fever streptococcus (I) were isolated in pure culture (see Plate VI for full particulars)

This was a very severe case of rheumatic fever and a large number of rheumatic nodules were present. The patient had had previous attacks and there was much cardiac damage present.

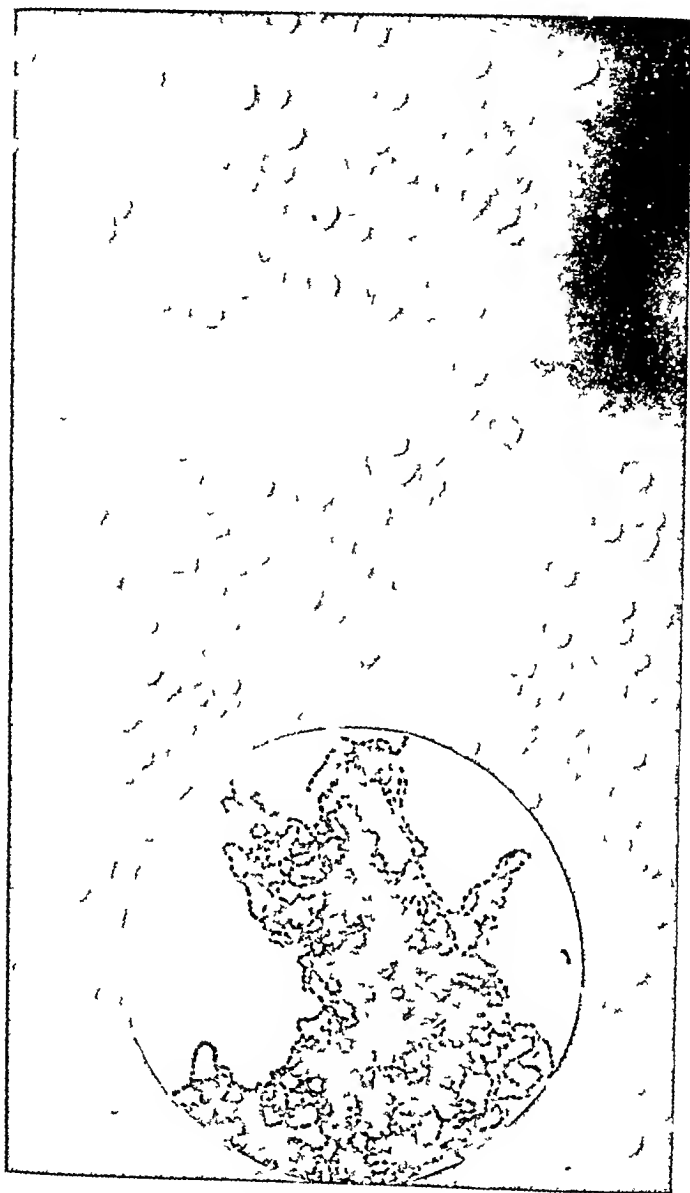


PLATE VI

Streptococcus (Viridans Type) Isolated from the Throat of Acute Rheumatic Fever (Case Campbell) (Pure culture)

Microphotograph of colonies on Crowe's chocolate blood agar after three days aerobic incubation at 37.5 C. Magnified 25 diameters.

The colonies are soft moist glistening heaped up with a whitish yellow zone of bleaching around them.

Iodine +- (That is they are changed to a light brownish color with Lugol's solution)

Broth Culture—Shows a copious white woolly deposit with a clear supernatant fluid

Difficult to emulsify

Inset Circle—Broth culture magnified 1700 diameters

SUGAR REACTIONS

Glucose +	Maltose +	Galactose +	Saccharose +	Lactose +	Mannite -
Dextrin -	Inulin +	Salicin -	Raffinose -	Litmus Milk +	
				Clot	

The typical appearance of these streptococcal colonies characteristic of rheumatic fever is shown on Plates III, V and VI. The colonies are as a rule somewhat spherical, smooth and glistening and are surrounded by a yellow zone of bleaching. When incubated for a period longer than three days they become more rough and opaque and tend to spread a little around their margins.

In both cultures as a rule they produce a flocculent deposit with a clear supernatant fluid. The deposit consists of long tangled skeins of streptococci. They ferment glucose, maltose, galactose, saccharose, lactose and produce acid and clot in litmus milk. Mannite is never fermented but occasionally dextrin.

They resemble very closely the streptococcus RFV 182 isolated by Birkhaug from a rheumatic fever nodule. (See *Annals of the Pickett Thomson Research Laboratories* 4, 1928, Plate 4, Figs. 4-6.) They are entirely different from the *S. cardiorhithridis* of Small and the nonmethemoglobin forming streptococcus of Birkhaug (see Plates I and II of this paper).

3 Pathogen Selective Cultures from the Throat. This method introduced by Cronin Lowe (1928) was adopted in two cases. The material from the throat swab was incubated with the patient's own blood for a period of twenty-four hours. Cultures from the blood were then plated on Crowe's medium and incubated for three days as usual. Plate V shows a microphotograph of a culture from a rheumatic fever throat made in this manner. Only two organisms were present, viz., *Staphylococcus aureus* and the typical colonies of the rheumatic fever streptococcus already described.

The second attempt at pathogen selective culturing from the throat of another rheumatic fever case was even more successful as the characteristic colonies of the streptococcus appeared almost in pure culture.

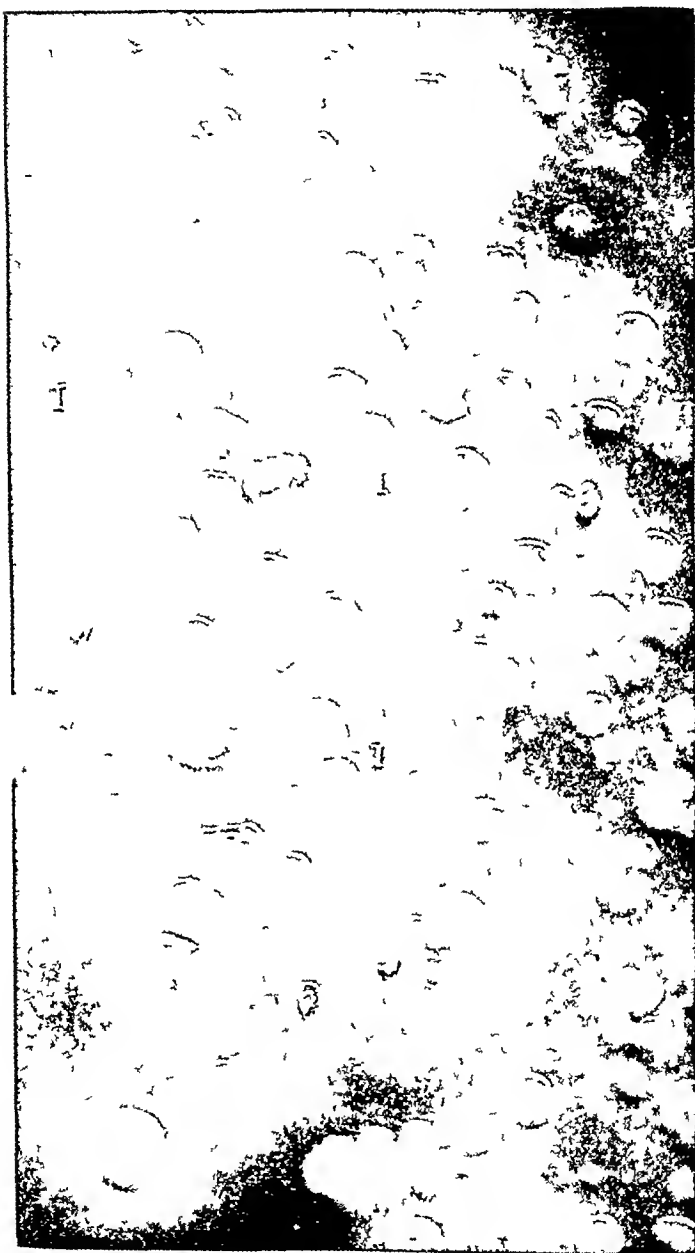
4 Tonsil Puncture Cultures. We believed that if cultures were made from the interior of the tonsils, we would be more likely to obtain purer cultures of the streptococcus than by the surface swab method. Dr. Gray Hill tried this method following the procedure of Pearse in eleven cases.

The results were, however, somewhat disappointing. In several instances the plates were overgrown with a mass of bacteria including the usual flora of the mouth.

The streptococcus characteristic of the rheumatic fever throat swabs except in a few instances where they were very numerous, was not nearly so much in evidence in the case of the tonsil punctures. In three instances hemolytic streptococci were obtained in the mixed flora and in one instance the Small Birkhaug type of streptococcus (see Plates I and II) were numerous.

5 Cultures from the Interior of Tonsils Enucleated During the Attack of Rheumatic Fever. Samples taken from the interior of tonsils enucleated shortly after an attack of rheumatic fever were inoculated on to Crowe's medium in the usual manner. In one of these large numbers of hemolytic streptococci were found as well as a few of the characteristic rheumatic fever streptococcus. In other cases were found hemolytic streptococci, the streptococcus of Birkhaug and Small and the characteristic viridans streptococcus.

6 Postnasal Cultures. Cultures were made from the nasopharynx in four cases but they showed nothing unusual. The chief organisms obtained from



• PLATE VII

this situation were gram negative diplococci of the catarrhalis type and streptococci of the pneumococcus type

7 **Urine Cultures** The urine in one instance was drawn off aseptically, centrifuged and the deposit inoculated on to plates of Crowe's chocolate blood agar. A *Staphylococcus albus* and a few diphtheroids appeared on the culture but no streptococci.

8 **Postmortem Material** There is great difficulty in obtaining post mortem material from early cases of acute rheumatic fever. As a rule the cause of death is heart trouble and this usually occurs long after the acute stages of rheumatic fever have passed.

Dr Gray Hill was able to obtain postmortem material for bacteriologic examination in three cases.

CASE "C"—Blood was taken on the third day after death. This gave a mixed growth of several organisms, among which was a hemolytic streptococcus.

CASE "S"—Cultures were taken from the mitral valve, pericardium, myocardium and heart blood, one day after death. Two varieties of streptococci were isolated from the mitral valve. One resembled somewhat the Birkhaug Small colorless streptococcus. The other varieties produced a flat spreading growth and caused a slightly greenish discoloration of the culture medium.

CASE "M"—The pericardium was full of seropurulent fluid at the post mortem and from this pus was cultivated a *Staphylococcus aureus* and two varieties of streptococci. Both varieties of streptococci were of the viridans type. One produced flat spreading colonies on Crowe's medium and was long chained. The other variety resembled the typical rheumatic fever species which we have found constantly in the throats of acute rheumatic fever cases (see Plates III to VI).

b *Further Remarks With Regard to the Characteristic Streptococcus Found*—If the streptococcus in question has some relation to the cause of rheumatic fever, one would expect to find it in every case and unless we are to assume that there are healthy carriers it should be absent from practically all other throats. Streptococci giving somewhat similar colonies, however, can be found in the throats of some normal children. From our investigations it would appear that several varieties of streptococci are capable of producing this type of colony, so it is necessary to investigate this matter

PLATE VII

Primary Culture from Measles Throat—(Case Cooper fourth day)

Microphotograph of the culture on Crowe's chocolate blood agar after two days anaerobic and one day aerobic incubation at 37.5 C. Magnified 25 diameters.

It will be noted that the majority of the colonies (1) are flat and translucent with a raised margin. They are Iodine - or +. These colonies have been found by Drs. D. and R. Thomson to be present in large numbers in all cases of measles so far examined. When isolated in pure culture they are found to give the following sugar reactions:

SUGAR REACTIONS

Glucose	Maltose	Galactose	Saccharose	Lactose	Mannite
+	+	+	+	+	-
Dextrin	Inulin	Salicin	Raffinose	Litmus Milk	
+	+	+	+	+	
or -	or -	or -	or -	Clot	

This measles streptococcus resembles very closely those strains isolated from cases of measles by the American workers Tunkill and Ferry.

It will be noted that the type of heaped up glistening viridans streptococcus found in the rheumatic fever cases (Plate III VI) are conspicuously absent.

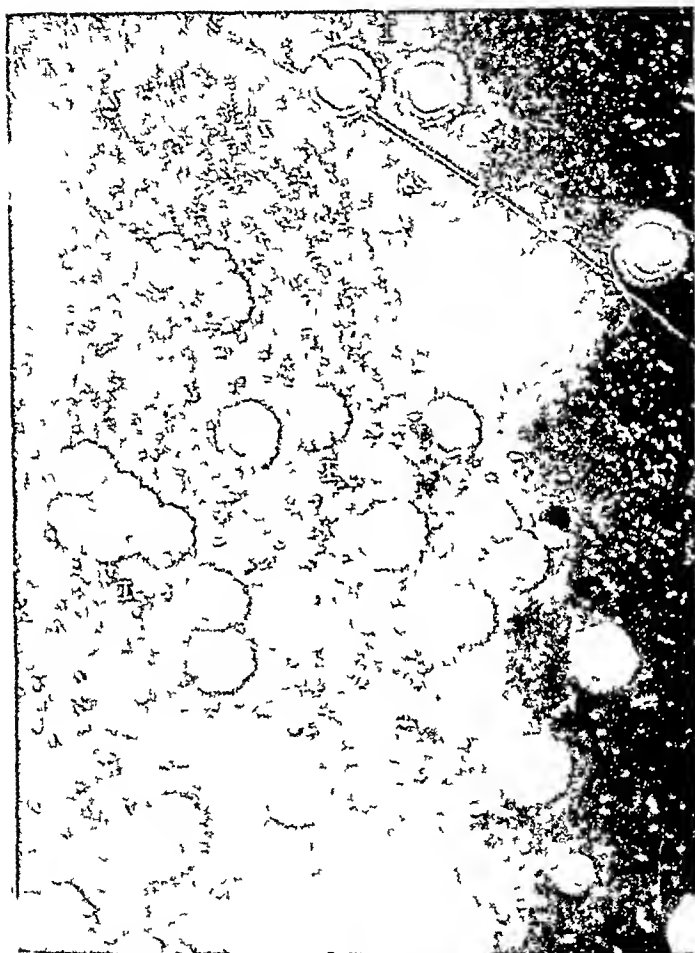


PLATE VIII

Primary Culture from Scarlet Fever Throat—(Case Birch third day)

Microphotograph of the culture on inspissated whole blood (modified Crowe's medium) after three days aerobic incubation at 37.5°C. Magnified 25 diameters

The predominant colonies (I) are the haemolytic *Streptococcus scarlatinae*. These colonies are greivish soft moist with no color around or under them. They are iodine—and haemolytic

NOTE—That the colony picture is very different from that obtained from the throat cultures of rheumatic fever measles etc

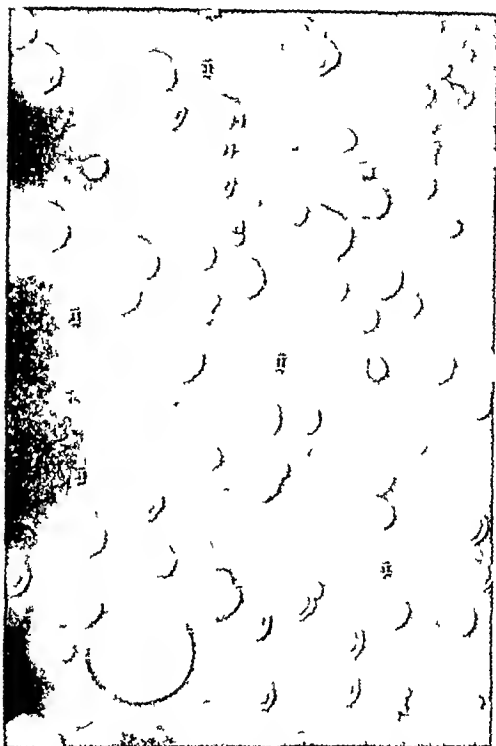


PLATE IV

Primary Culture from the Throat Severe Influenza.—(Case Webb)

Microphotograph of culture on Crowe's chocolate blood agar after three days aerobic incubation at 37° C (Magnified 25 diameters)

The predominant colonies (I) were whitish soft moist heaped up (cottage loaf type) with a greenish yellow discoloration of the medium around. This type of viridans streptococcus which will be noted is quite different from the types found in rheumatic fever and in measles was present in all the cases of influenza examined though not in such large numbers as in this very severe case.

This patient developed endocarditis with emboli and eventually died

SUGAR REACTIONS OF THE INFLUENZA STREPTOCOCCUS (I)

Glucose	Maltose	Galactose	Saccharose	Lactose	Mannite
+	+	+	+	+	-
Dextrin	Inulin	Salicin	Raffinose	Litmus Milk	
+	-	-	-	+	
				Slight clot	



PLATE X

Primary Culture from the Healthy Throat of a Nonrheumatic Child

Microphotograph of culture on Crowe's chocolate blood agar after three days incubation at 37.5 C. Magnified 25 diameters

Colonies (I) which are of the Birkhaug-Small type are numerous although this child gave no history or any evidence of rheumatism. The small viridans colonies (II) appear to be of a different type from those found in rheumatic fever throats

very carefully by employing the usual other tests for identification such as sugar reactions, skin tests, agglutination tests, etc. These tests are at present in progress but we have not yet done sufficient work to come to any definite conclusion. We wish it to be understood, therefore, that although we find this type of streptococcus constantly present in the throats of rheumatic fever patients, we do not yet claim that it has any real causal relation to the disease. Its persistent frequency, however, is sufficiently striking to compel us to regard it with strong suspicion.

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THE NUTRITIONAL FACTOR IN CHRONIC ARTHRITIS*

By A. A. FLETCHER M.B. (TOR.), TORONTO

IN MOST cases of chronic arthritis, the onset appears to be initiated either by infection, by some form of trauma or strain, or by the menopause. Those associated with infection are usually cases of rheumatoid arthritis, and those with trauma and strain or the menopause, osteoarthritis. But there is much doubt as to how essential and how important any of these so-called exciting factors may be as causes of the disease. In those cases which appear to be infectious microorganisms are not recovered with any certainty. When isolated they are as a rule of low virulence and do not conform to any specific type. Most observers report that they are unable to reproduce chronic arthritis in experimental animals by the injection of these bacteria. Much uncertainty also exists as regards trauma and the precipitating traumatism may be of a trivial nature. And while it is true that arthritis commonly develops at the time of the menopause, this change by itself could scarcely result in joint disease.

Considerations such as these lead to the belief that some state of ill health on the part of the patient has preceded the onset of the disease. This state has been vaguely designated under terms such as "constitution" or "diathesis." From a clinical standpoint it is apparent that many factors contribute to the development of this state, such as fatigue, climate, environment, occupation, heredity, metabolism, worry, anxiety, previous disease, age, sex, diet and nutrition. This paper has to do with the nutritional and dietetic factors.

A relationship is generally recognized between the digestive system and the development and course of chronic arthritis. Some disturbance of this system such as constipation, diarrhea or putrefaction, may precede the onset of joint disease or parallel its severity. Relief of arthritic symptoms may be brought about by changes in diet, by the use of colon irrigations, by the administration of laxatives or intestinal antiseptics, or by the implantation of acid forming organisms and these measures calculated to correct some digestive disturbances are widely used at the present time. Goldthwaite¹ and his colleagues, Lane,² Rea Smith,³ and others have directed attention to certain changes found in the abdominal viscera: visceroptosis, intestinal stasis and bowel atony. Based on the belief that these mechanical disturbances were of fundamental importance many therapeutic measures have been carried out which include the application of belts, postural re-education, plastic abdominal operations, and even partial colectomy, and at times these have resulted in marked improvement of the arthritis.

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But dietotherapy in arthritis has meant many diets some of them based upon conflicting and unscientific data. The uric acid and autointoxication theories, in particular, have resulted in undue and harmful restriction of meat and glandular foods. For many years however, Pemberton⁴ has insisted upon the value of limitation of the diet lightening in this way the load upon both the digestive tract and upon general metabolism. He lays great stress upon the restriction of carbohydrate. Some years ago we observed the beneficial effects of this method of treatment and we reported that carbohydrate restriction was most effective when vitamins were added. It was suggested that in arthritis excess carbohydrate aggravates latent vitamin deficiency and to this state of malnutrition was due the susceptibility to bacterial or toxic invasion.

In a further study of the influence of high vitamin low carbohydrate diet in arthritis, special observation⁵ has been made of the bowel disturbances. In the past it has been said that they were developmental or constitutional in origin or the result of some acquired defect. It is likely that many factors determine the position of the abdominal viscera and influence the tone and motility of the bowel. Our own observations indicate that they are more particularly dependent upon the nutritional state and dietetic life of the patient. In 1921, McCarrison⁷ reported that he was able to produce bowel atony and mucous membrane degeneration in the colon of monkeys by feeding unclayed diets high in carbohydrate and it appears that the bowel changes found in patients with chronic arthritis are structurally and causally of the same nature.

These colon disturbances may be demonstrated clinically by an x ray examination following a barium enema.⁸ They include loss of tone, decrease or absence of haustral markings and increased length. The loss of tone may be most marked in or confined to the cecum and ascending colon. At other times it may involve most of the viscera, so that with the absence of haustration the bowel presents a large sausage shaped appearance. The atony may also result in shapelessness of the bowel, and the increased length produces redundancy and looping. Under the influence of high vitamin low carbohydrate diet, tone improves, haustral markings increase and become regular and the colon tends to regain its normal shape and contour.

In the formation of these diets foods high in vitamin are used as far as possible and Vitamin B is given liberally in the form of wheat germ, wheat germ extract, or various forms of yeast. Wheat germ is most effective in bringing about this improvement in the colon. It may be given up to one ounce or one and one half ounces a day. Arrangement of the diet is also essential in this treatment. Protein is supplied as meat, fish, fowl, eggs, liver, and milk up to 50 or 60 gm a day. Carbohydrate is reduced to 50 to 75 gm given largely as vegetables and fruits. Fat has been allowed to make up the caloric requirement. It should be given largely as cream or butter. Some individual adjustment in diet may be necessary. There is much variation in protein tolerance especially when putrefaction has set in. In such patients a short course of dieting with milk alone may be carried out. On the other hand arthritic patients often show considerable anemia, and pro

longed protein restriction is inadvisable. Patients who are overweight may, with benefit, be given diets well under the basal requirement. In those underweight, the calories should be given in the form of cream and butter to exceed a maintenance level. The changes which take place in the colon are not dependent upon bulk or roughage. Some patients do best on a bland diet others on diet large in volume. Wheat germ may aggravate colitis if it is present, and Vitamin B is better borne as yeast or yeast extracts.

This program of dietetic treatment materially modifies the course of the arthritis and most benefit is seen in patients who show colon disturbances. Observation has been made under careful control, and diet alone in some cases may result in quiescence of the disease. There is, at the same time, improvement in the general health of the patient. The patient with arthritis is often a chronic invalid and malnutrition may be part of his physical asthenia. Increase in weight and strength, more resistance to fatigue, increased appetite, better bowel function may be part of the general improvement. In chronic arthritis a disturbance in capillary flow may be present. This, many believe, is of basic importance (Rowntree⁹). Many physiotherapeutic measures probably act through their influence on this system. This disturbed capillary flow may disappear under a course of dietetic treatment and the extremities cease to be damp and cold.

Treatment by diet should not be used to the exclusion of other measures. Clinical experience indicates a multiple etiology of arthritis, and treatment should embrace many measures calculated to combat the various remote and immediate causes. Some three or four hundred patients have been treated by this high vitamin low carbohydrate program, and it may be said that in many no other method of treatment has been so effective. In 60 to 65 per cent of the cases the colon showed a radiologic appearance which was considered abnormal. In these cases dietetic measures are almost always beneficial. Even in patients without abdominal disturbances treatment by diet may prove a valuable adjunct to other measures adopted.

The probable nature of the disturbed nutritional state in chronic arthritis requires some discussion. Human diets are rarely deficient in one constituent only, and it appears likely that the condition is not comparable to the specific vitamin deficiency diseases of experimental animals. McCarrison, in producing lesions of the colon by deficient and unbalanced diet, pointed out their complex etiology. He attributed these changes especially to Vitamin B deprivation, but he further emphasized the part played by lack of balance in the diet and particularly to carbohydrate excess. Deficiency of other vitamins also interferes with the normal growth of the digestive tract. Cramer¹⁰ has shown that development of the mucous membrane of the bowel is dependent upon adequate Vitamin A. In rats fed on diets partly deficient in this vitamin, atrophic and destructive changes were seen in the intestinal mucous membrane leading to the invasion of microorganisms. The motor disturbances of the bowel are associated especially with Vitamin B deficiency, probably the thermolabile or antineuritic B₁.

The diets used in treatment involve two general principles. The liberal use of the so-called protective foods, those which are high in vitamin, and

second, the restriction of certain foods which favor deficiency disease, namely, the carbohydrates. Previous to McCarrison's observations Funk¹¹ had found that the ease of producing Vitamin B deficiency varied directly with the amount of carbohydrate administered, and Mellanby¹² states that the amount of cereals in the diet will determine the severity of Vitamin D deficiency disease. To replace the carbohydrate, fat may be given freely and this is in keeping with the experiments of Evans and Lepkovsky¹³ who find that fat has Vitamin B sparing properties. General improvement in the diet is essential in treatment. Adequate protein of good biologic value and foods high in all vitamins and also inorganic elements should be supplied. Cod liver oil is often beneficial. The patient should be brought to a state of optimum nutrition.

Furthermore, the recovery period is likely to be slow. These colon changes antedate the arthritis as far as we know and clinical histories may reveal long standing gastrointestinal disturbances in function. Faulty nutrition does its greatest harm during growth and if this is chronic goes on to permanent structural damage as a result only partial convalescence may be possible. Sometimes improvement in the colon disturbance takes place in a few weeks, but more often it is a question of months. Some of these patients appear to be incapable of returning to ordinary diets. They may be permanently intolerant of carbohydrate, and it is advisable to continue more or less indefinitely these principles of dietetic treatment.

Many patients with chronic arthritis have foci of infection and the onset of the arthritis is related to active focal infection. This is true for those with colon disturbances and for those without. In patients with these colon disturbances dietetic treatment influences not only the arthritis, but also the liability to infection, and it appears likely that in these patients faulty nutrition has led to a state favorable to the development of infection. Animal experiment offers much support to this opinion. Vitamin A or B deficiency is particularly liable to be associated with infection and in each case the deficiency disease itself may be an infectious process. Mellanby¹⁴ would have Vitamin A the antiseptic vitamin. Variations in protein administration also influence susceptibility to infection. Of special interest in this connection is the work of Cramer¹. He subjected rats to prolonged non specific vitamin underfeeding and in these animals avirulent organisms became pathogenic. This is comparable to the phenomenon of arthritis when organisms of low virulence bring about profound invalidism. The liability of the patient with chronic arthritis to develop focal infection is as much an expression of his chronic ill health as a cause of it, and this tendency to run chronic infections is influenced by corrective dietetic measures.

Visceroptosis and intestinal stasis, as well as atony, are associated with this state of faulty nutrition. Under a course of dietetic treatment the cecum or transverse colon may be lifted up out of the pelvis, and the splenic and hepatic flexures rise three or four inches. It is possible that visceroptosis is due to other causes but from an examination of the x-ray plates, it is evident that the position of the bowel in the abdomen can be much modified by these diets. While prejudicial to the health of the patient, it is doubtful

whether these mechanical disturbances of the abdomen, as revealed by x-ray, represent in themselves primary causative agents in arthritis. A marked degree of stasis and atony may be seen without arthritis as, for example, in Hirschsprung's disease. Other factors which are associated with the bowel disturbances are probably of greater etiologic importance. These are first, general faulty nutrition leading to infectious or premature degenerative disease, and second, damage to the mucous membrane of the bowel, leading to increased permeability. In many patients the large bowel is the site of an infectious or catarrhal process evidenced clinically by colitis and by tenderness throughout the colon or in the cecal region. It seems highly probable that the diseased colon may at times be the focus of the infection or toxic agent causing the arthritis.

These colon disturbances have been observed in patients with rheumatoid arthritis and with osteoarthritis, and in some with arthritis of the menopause. In all these cases dietary measures have proved beneficial. This supports the view, which is widely held, that whatever the exciting cause may be, the different forms of chronic arthritis have some causal background. Opinion is expressed that faulty nutrition is the most frequent and most important basic factor in the development of this disease, and of the various factors that go to make up the predisposing state, none is more frequent or more important than chronic faulty nutrition.

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MEDICAL ARTS BLDG

TRAUMATIC ARTHRITIS AND THE MECHANICAL FACTORS IN HYPERTROPHIC ARTHRITIS*

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INTRODUCTION

CHRONIC arthritis is now recognized as probably the greatest cause of human morbidity and during recent years this disease or condition has attracted the attention of investigators throughout the world. For the most part attention has been focused upon infection as the etiologic factor in the disease. Among recent American authors Forkner and Shands,¹ Cecil,² Burbank,³ and Key⁴ have all obtained organisms from the blood or joints of patients with chronic arthritis and have endeavored to prove that these organisms are the cause of the disease. Unfortunately each of the above investigators has obtained one or more different organisms and no one has yet obtained acceptable proof that any organism is the cause of any of the conditions commonly called chronic arthritis, so the etiology remains obscure.

At this point it should be definitely stated that chronic arthritis is not a clinical entity but embraces a considerable number of more or less similar conditions. They have been abundantly but not as yet satisfactorily classified as anyone who has studied large series of cases is aware of his own inability to fit many of the cases into any of the various classifications which are to be found in the literature. However most of the cases fall into one of two groups (1) atrophic proliferative, rheumatoid infections or ankylosing arthritis or arthritis deformans and (2) hypertrophic, degenerative senile metabolic or osteoarthritis and this is also called arthritis deformans. In this paper the terms atrophic and hypertrophic arthritis will be used. But there are of course, many cases which do not fit into either group and other cases which appear to be a mixture of both types in one individual. Finally after studying the subject rather intensively during the past ten years I find cases which are mixed atrophic and hypertrophic and other borderline cases which I believe are either atrophic or hypertrophic but I am not sure which.

The pathologic picture and clinical course of atrophic arthritis resemble those of an infectious disease and for this reason the impression that some or perhaps one of several as yet unknown organisms is the cause of the condition has been steadily gaining favor during the past few years. In hypertrophic arthritis on the other hand the pathologic picture and clinical course resemble those of a joint which is reacting to injury caused by a long continued series of insults which may be either mechanical or chemical in nature.

This paper will deal with hypertrophic arthritis and the atrophic form of the disease will not be considered. It will include a short review of the results

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obtained by efforts to reproduce the disease in experimental animals, a brief discussion of traumatic arthritis, and a consideration of certain mechanical factors which I believe influence the origin and progression of clinical hypertrophic arthritis

THEORIES AS TO THE ETIOLOGY OF HYPERTROPHIC ARTHRITIS

One of the fundamental questions in the problem of hypertrophic arthritis is "Where does the disease begin?" The pathologists of the last century found various answers to this question in the specimens which they examined. Rokitsky concluded that the primary change was an inflammatory osteoporosis in the subchondral bone which was followed by a sclerosis with secondary degeneration of the cartilage. Ziegler believed that the primary pathologic change was an atrophy of the subchondral bone and that this was followed by a loosening of the articular cartilage. Weber, on the other hand regarded the cartilage changes as secondary to an abnormal calcification in the border zone between the bone and cartilage. Hein considered chronic arthritis a nutritional disturbance in the hyaline cartilage and Rimmann believed that the joint changes were the result of some chemical alteration in the tissue fluids caused by tuberculosis, syphilis, cachexia, or arteriosclerosis. Volkmann believed that the disease was primarily a chronic inflammation of the synovial membrane and that this was followed by a secondary ossifying hyperplasia of the articular cartilage.

From the above paragraph it is evident that the study of pathologic material has led profound students of bone and joint pathology to widely different conclusions and that the subchondral bone, transition zone, articular cartilage, synovial membrane, and tissue fluids have each been considered the starting point of the disease process.

More recent authors have endeavored to explain the etiology of the disease. One of the most plausible of these is Weichselbaum⁵ who made an exhaustive study of the joints of senile individuals and concluded that chronic hypertrophic arthritis was merely a high degree of these changes in the involved joints. But no explanation is offered as to why the changes occur early in certain joints and in certain individuals.

Wollenberg⁶ modified the theory of Hein that the disease is due to a nutritional disturbance in the joint cartilage to the extent that this nutritional disturbance is in the nature of an ischemia due to local arteriosclerosis and that the arthritis is primarily a vascular disease in which the joint changes are secondary to the local vascular sclerosis.

Axhausen,^{7, 13} as a result of some experiments to be mentioned below, concluded that cartilage necrosis is the primary cause of the disease and that the presence of dead cartilage in the joint exerts some influence, possibly chemical in nature, which leads to the development of the classical disease picture.

Preiser⁸ from an extensive study of clinical cases, concluded that incongruity of the joint surfaces results in local cartilage degeneration from relatively abnormal pressure and anemia, and that this leads to the further

development of the symptom complex of hypertrophic arthritis. Likewise, Nichols and Richardson,⁸ from their pathologic studies, regarded long continued abnormal pressure with resultant cartilage degeneration as an important factor in the etiology of many of their cases.

Bencke¹⁰ is usually considered to be the founder of the mechanical functional theory. From his studies on spondylitis deformans he concluded that the primary change was a degeneration of the intervertebral discs and that the changes in the vertebrae were the result of the continuous mechanical trauma incident to normal use in the presence of the degenerated discs. This theory has been slightly modified by Poinmer¹¹ to the effect that the primary change is a loss in elasticity of the articular cartilage and that the disease is the result of repeated trauma to the underlying and now insufficiently protected bone.

Goldthwaite¹ has been the chief exponent of the mechanical theory in this country and attributes the development and progress of the disease to faulty body mechanics and abnormal strain on the involved joints. His great contribution has been the emphasis placed upon the patient as a whole rather than upon the local manifestations of the disease and his insistence upon the correction of faulty bodily mechanics, not only to relieve local strain upon the involved joints, but also to improve the general physiology of the patient and especially as a means of bettering the function of the abdominal and thoracic viscera.

EXPERIMENTAL HYPERTROPHIC ARTHRITIS

During the past twenty years a number of investigators have produced the changes characteristic of hypertrophic arthritis in various ways. In this paper we are primarily concerned with those in which the changes result from a definite injury to the joint. Among the first of these were the experiments of Axhausen⁷ who killed a part of the joint cartilage in the knees of experimental animals by the application of iodine or ammonium hydroxide and produced pathologic changes which resembled those present in advanced hypertrophic arthritis. A few years later in order to eliminate the chemical factor, he produced similar changes by cauterizing the surface of the cartilage with an electric needle. He believed that the development of the arthritis was due to the presence of the dead cartilage in the joint. (Axhausen¹³)

Wollenberg,¹⁴ in attempting to prove his vascular theory, passed silk ligatures through the tissues around the patella in order to shut off the blood supply and after six months he found *degenerative and hypertrophic changes* in this bone. He interpreted the results as being due to local ischemia and thus accepted his experiments as proof of his vascular theory.

Axhausen and Pels¹⁵ repeated Wollenberg's experiments and believed that the arthritic changes were due to the cartilage necrosis rather than to the ischemia.

Recently Pemberton and Goldhaft¹⁶ have again repeated the experiment and adopted Wollenberg's interpretation of the results.

Kroh,¹⁷ stimulated by the clinical observations of Preiser, resected one condyle of the femur in rabbits and found that arthritic like changes devel-

oped in the joints His joints were not studied histologically, but he believed that the experiments proved Pieser's theory that arthritis is due to incongruity in the joint surfaces

Manteuffel¹⁸ produced arthritic-like changes by freezing the knee joints of guinea pigs with an ether spray and by passive hyperemia He did not consider the arthritis as being a result of vascular sclerosis, but thought that it was due to some other and as yet obscure cause

Sury,¹⁹ by repeated forcible manipulations or by percussion with a reflex hammer, produced degeneration and loosening of the cartilage with vascular sclerosis and ossification He believed that he had produced a true traumatic arthritis

Mullei,²⁰ by suturing the humerus of rabbits to the scapula, immobilized the shoulder in such a manner that the tendon of the biceps remained constantly pressing upon the cartilage of the humerus Under these conditions the cartilage became eroded and arthritic-like changes developed in the bone From this experiment he concluded that the arthritis was due to abnormal pressure on the cartilage

Wchner²¹ resected the patella in a series of rabbits and found that arthritic-like changes resulted He believed that his experiments showed that the arthritis was due to abnormal use of the joint

Burkhardt²² repeated Arlhaussen's experiments with carbolic acid and iodine and added the factor of immobilization in order to determine whether or not function was concerned in the development of the pathologic picture of arthritis deformans In the joints which were used the picture of arthritis deformans developed in from three to five months while in the joints which were immobilized the cartilage remained intact for a long time and was gradually replaced by connective tissue and new cartilage which in some instances caused the joint to be filled with a cartilaginous connective tissue and tended to produce ankylosis with a picture resembling that of atrophic or gonorrheal arthritis He found that villi developed in used joints while pannus developed in the immobilized joints and concluded that arthritis deformans is a regeneration phenomena which is due to cartilage injury and takes its anatomical characteristic from the mechanical function of the involved joint

Carey (E J personal communication) tells me that he has found arthritic changes in the joints of dogs which were exercised over long periods in a treadmill

I have produced mild arthritic like changes in the knee joints of rabbits by the injection of mild irritants (Kev²³) and more recently I have shown that the pathologic picture of hypertrophic arthritis can be produced in the knee joints of rabbits by resecting a small rectangle of cartilage from the patellar surface of the femur (Kev²⁴)

A simple method of testing the mechanical theory would be to create an abnormal strain on a joint which was in other respects perfectly normal Thus I have attempted to do by the production of knock-knee in a series of twelve young rabbits The method was to anesthetize the animal and simply

bend their legs outward at the knee over the edge of the table. By repeating the manipulation at weekly intervals over a period of weeks it was possible to obtain permanent valgus deformities of about 30 degrees. Seven of these rabbits grew to adult life and in each of these there was definite chronic



Fig 1—Photograph of lower end of the femurs and soft parts of rabbits in which valgus deformities were produced by manipulation. All showed injuries to the bones or ligaments and the arthritis was proportional to the injury.

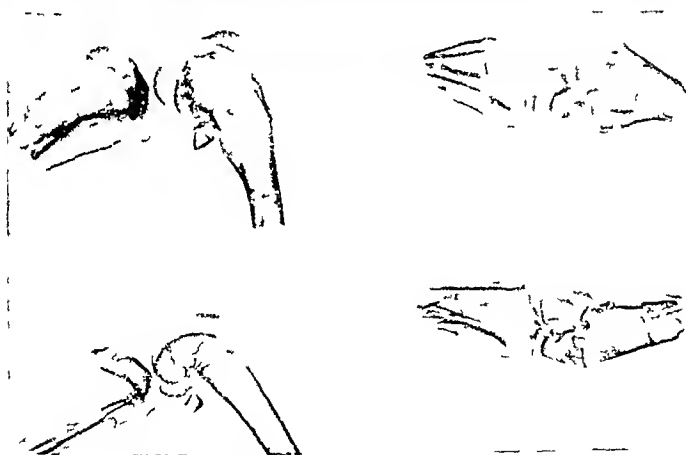


Fig 2—X rays of knees of rabbits illustrated in Fig 1

arthritis of the hypertrophic type with osteophytes around the cartilage margin, more or less hypertrophy of the involved bones and some hyperplasia of the synovial membrane. The most marked changes were found in those in which the patella had been displaced outward and rested upon the lateral surface of the femoral condyle. However the value of these experiments is lessened by the fact that all of the knees showed evidence of definite articular

lar damage which had occurred at the time of the manipulation. Either the lower end of the femur had been fractured, the femoral epiphysis had slipped, or the crucial ligaments had been ruptured. In other words in these animals what was produced was traumatic arthritis from a disorganization of the



Fig 3—Hallux rigidus due to an old contusion of the articular cartilage. Note the almost complete absence of the cartilage at the metatarsophalangeal joint

joint or cartilage and bone injury and not chronic arthritis as a result of faulty mechanics in weight-bearing (Figs 1 and 2)

From the foregoing it is evident that the pathologic changes characteristic of hypertrophic arthritis can be produced in many different ways

TRAUMATIC ARTHRITIS

Let us now consider trauma as a factor in the production of chronic arthritis in man. The injury may be of several types (1) a single severe injury to the joint cartilage, (2) repeated mild trauma to the joint cartilage,

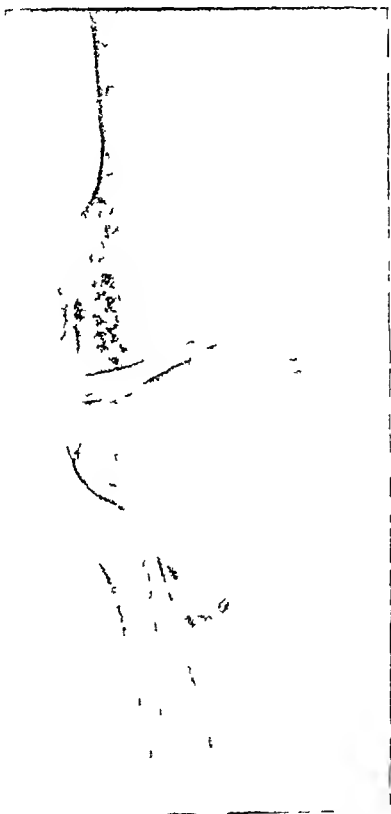


Fig 4—Traumatic arthritis following an old injury to the internal semilunar cartilage and anterior crucial ligament. Note the narrowing of the joint space, roughening of the cartilage and the osteophytes over the internal condyle.

(3) disorganization of the mechanics of a joint (4) faulty weight bearing on account of bony deformity so that use brings about repeated injury to the joint surface, (5) gradual deformity of a joint by abnormal pressure.

1. An example of an arthritis resulting from a single injury to the joint cartilage is shown in Fig 3 which is the x ray of the toe of a man fifty one

years old who violently kicked a toe while running after a ball twenty-five years ago. Apparently there was no fracture as there was no prolonged disability, but the toe was sore, swollen, and painful on motion for a few days. The metatarsophalangeal joint has been the site of occasional pain off and on for years and during the past five years the symptoms have grown steadily worse. Examination revealed a hallux rigidus with only a few degrees of motion and pain at the limits of motion. The x-ray shows evidence of almost complete absence of the articular cartilage and considerable production of new bone around the joint. In other respects the patient is normal and there is very little arthritis in any other joint. I believe that the arthritis in this joint is due to the old injury with contusion and degeneration of the cartilage. This belief is strengthened by the fact that I have seen three other cases of hallux rigidus with almost identical histories and physical findings.

2 Fig 4 is an x-ray of a knee joint of a woman who had an injury to her internal semilunar cartilage and anterior crucial ligament twenty-five years ago. From time to time since the original injury the knee has locked or caught on her while walking or exercising and on some of these occasions the joint became swollen and she was disabled for some weeks. At one period she was free from symptoms over a period of fifteen years. During the past two years the pain and catching have been largely limited to the region of the posterior portion of the external semilunar cartilage.

At operation the internal semilunar was found to be markedly degenerated, all that was left being a thin outer rim with several loose fringes of fibrous tissue hanging into the joint. The cartilage over the internal condyles of both bones was roughened and fibrillated and partly eroded, and the cartilage over the external condyles showed slight roughening. The external semilunar was abnormally loose, the anterior crucial ligament was absent, and there were numerous osteophytes around the articular margin of the femur and the fat pad and synovial membrane were moderately thickened.

I believe that the repeated mild injury caused by the loose or torn internal semilunar cartilage and torn crucial ligament caused the degeneration of the cartilage of the internal condyle and that this resulted in the osteophytes and synovial thickening.

3 Chronic arthritis as a result of disorganization of the mechanics of a joint is all too frequently seen after joint fractures in which the fragments have been imperfectly reduced and permitted to unite with more or less inexact restoration of the normal contours of the articular surfaces. A very familiar type is the traumatic arthritis of the knee seen after compression fractures of one or both condyles of the tibia. If the depressed fragment is large a genu valgum or varum may result and the insult of the abnormal weight-bearing is added to that of the inequality in the cartilage, but if the fragment is small the line of weight-bearing is not disturbed. However, even with a small portion of the lateral condyle depressed only $\frac{1}{8}$ of an inch I have seen chronic arthritis develop in the knee of a man thirty years of age and lead to so much disability that an excision and arthrodesis of the joint would be preferable.

Other shining examples are fractures of the head of the radius or of the neck of the radius with displacement of the head. In many of these cases early removal of the radial head will prevent the development of the arthritis and give the patient a useful painless elbow. A very striking example of this type of arthritis is seen in the hip after a Legg Perthes disease in childhood or a slipping of the upper femoral epiphysis in adolescence. In the former the femoral head and acetabulum are deformed and in the latter the displaced head of the femur no longer fits in the acetabulum. Practically all of these patients have pain and limitation of motion in the hip after they



Fig 5—Severe hypertrophic arthritis in a woman seventy years old in whom a genu valgum had been present since childhood. Note the complete disappearance of the cartilage, eburnation of bones and osteophytes over the external condyle.

reach adult life, and it is my belief that these mechanical defects in the hip acquired in early life are the cause of most of the so called *Malum Coxæ Senilis* (Key²⁵). Frequent examples are seen in the ankle and subastragloid joints after fractures at the ankle or of the os calcis and others are seen at the wrist after typical extension and compression (Colles) or other types of fractures of the lower end of the radius.

I have also seen traumatic arthritis develop in a joint after a fracture which had been apparently perfectly reduced but as a rule the degree of disability and arthritis vary directly with the amount of displacement especially in weight bearing joints.

4 A good example of an arthritis developing in a joint which has been subjected to prolonged abnormal strain is shown in Fig 5 It is an x-ray of the knee of a woman seventy years of age who had had a rather severe bilateral genu valgum since childhood Note the disappearance of the cartilage over the external condyles (points of abnormal pressure) and the marked arthritic changes throughout the joint

In extraarticular fractures particularly of the femur or bones of the leg which are permitted to unite with deformity the knee and ankle joints are subjected to abnormal strains and in early adult life tend to develop a



Fig 6 —Arthritis of the knee in which a valgus deformity followed an old injury Note the eburnation and osteophyte formation on the external condyles

chronic arthritis which is almost identical with the hypertrophic arthritis of late adult life except that it affects only the involved joints Fig 6 is an x-ray of a man thirty-five years old who had been kicked by a horse fifteen years previously with probably a fracture of the lower end of the femur He had a valgus deformity of 45 degrees and a compensatory varus deformity at the ankle with moderately severe hypertrophic arthritis in the knee

5 The classical example of an arthritis developing as a result of long continued abnormal pressure is seen in the arthritis of the first metatarsophalangeal joints which is present with hallux valgus and bunions (Fig 7) The deformity is usually the result of short shoes and the (bunion) hypertrophy of the metatarsal head is partly the result of abnormal pressure from

the shoe, partly of irritation at the insertion of the internal lateral ligaments caused by the toe being pushed outward and partly the result of the arthritis in the joint which in some as yet unknown manner causes overgrowth of bone. That this last factor may be important is evident from the frequent lipping and exostoses which are seen on the dorsal surfaces of the involved bones.

It is thus evident that various types of intra-articular injuries or mechanical insults may result in chronic arthritis.

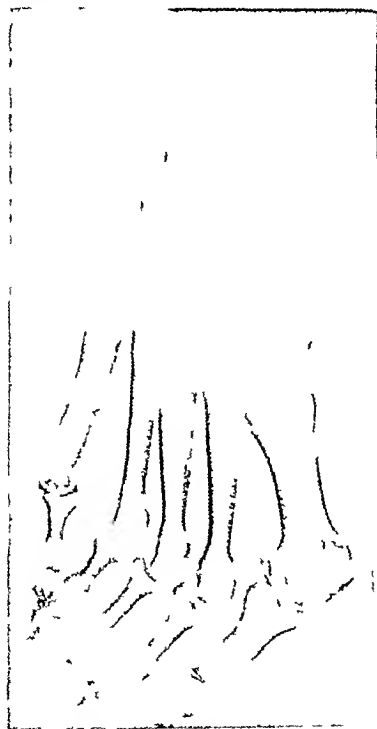


Fig 7—Typical bunion formation. Note the disappearance of the cartilage over the first metatarsophalangeal joint and the newbone formation around the head of the first metatarsal.

CLINICAL HYPERTROPHIC ARTHRITIS

Pathologically this disease is characterized by degeneration of the cartilage and eburnation of the bone over the bearing surfaces of the joints with the production of osteophytes around the articular margins and a variable amount of hyperplasia and infiltration of the synovial membrane and sub-synovial tissues.

The disease comes on after middle life and is particularly apt to affect the heavy type of individual and to be localized in the large weight-bearing joints and in the terminal interphalangeal joints of the fingers. It is more common among people who have performed much hard manual labor or who have static deformities which have placed unusual strain upon the involved joints.

One of the most interesting features of the disease is the fact that often there is no apparent relation between the extent of the pathologic changes

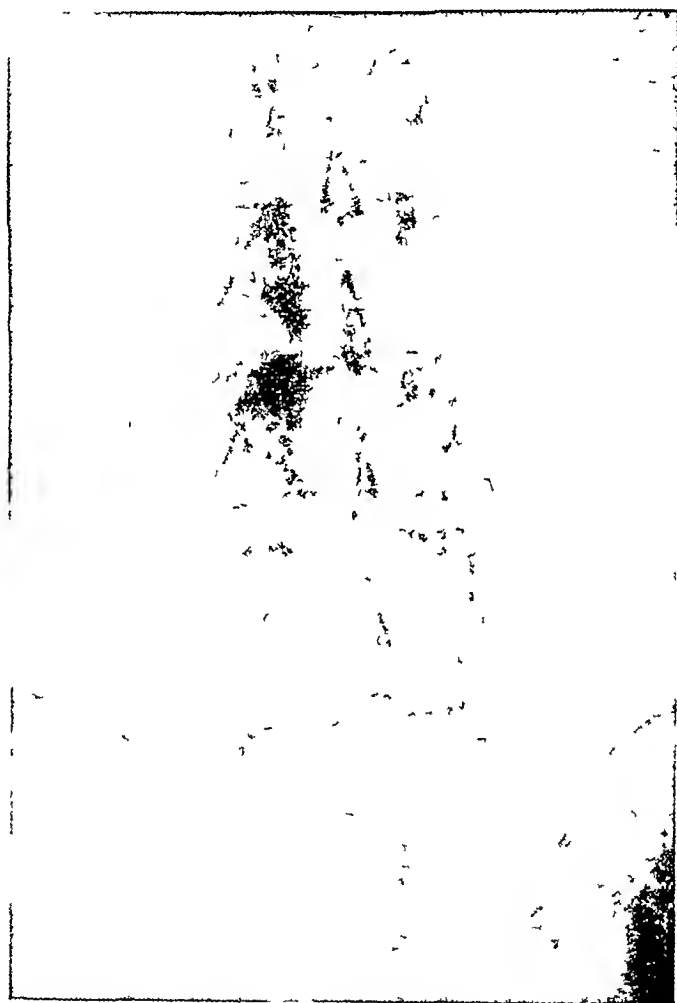


Fig. 8—Typical hypertrophic arthritis of the lumbar spine. Note the relatively broad flat vertebral bodies and the osteophyte formation at their margins.

and the severity of the clinical symptoms. In many cases extensive pathologic changes may be present in a joint over a long period of years and cause practically no symptoms and the patient may not be aware that there is anything wrong with his joints. On the other hand another patient may have considerable pain and disability in a joint which is practically normal to x-ray and clinical examination. Not only are the joints painful, but there

may be pain and tenderness over the adjacent bones particularly along the crest of the tibia in arthritis of the knee. Generalized muscular pain and stiffness are also frequent in patients in whom the joints are causing symptoms.

In many cases the symptoms vary with the weather and these patients usually feel worse with a fall in barometric pressure. In others the symptoms come and go without rhyme or reason and occasionally a mild injury to a joint may inaugurate a long period of pain and disability.

In examining patients with hypertrophic arthritis we are accustomed to look for static defects which may be a factor in the etiology of the disease. And quite frequently we find such defects. A common example is seen in the heavy women over fifty years of age with hypertrophic arthritis in the knees. In such patients the feet are usually pronated and the knees show a slight valgus deformity. We believe that one important factor in the development of the condition in these patients is the faulty mechanics of the knee joint with abnormal strain on the internal lateral ligaments and abnormal pressure on the external condyles with consequent injury to the articular cartilage. The abnormal strain and pressure are particularly evident in walking as the foot and ankle are turned outward and as the knee is flexed the inner side of the joint tends to open with consequent irritation at the sites of attachment of the internal lateral ligament and abnormal pressure on the external condyles.

Much the same mechanism is at work in the spines which are the subject of this type of arthritis. The vertebral bodies in these spines are usually of the broad relatively short type and due to their shape the amount of motion between the bodies of the vertebrae is much greater for a given amount of bending than occurs in a spine with relatively narrow long bodied vertebrae (Fig. 8). As a result the amount of motion in these heavy type spines is less than is normal for the slender type spine and the performance of a given amount of motion imposes a greater strain on the intervertebral ligaments on the convex side and abnormal pressure on the discs on the concave side of the curve. To this chronic irritation the bone responds by the production of the osteophytes around the vertebral margins which are the characteristic feature of the disease. In addition to the handicap mentioned above the spines of the heavy type individuals are usually placed under abnormal static strain as these people tend to develop pendulous abdomens, and in order to support the abdomen, they lean backward at the lumbar and lumbosacral regions with the development of an abnormal lumbar lordosis and a later compensatory dorsal kyphosis and round shoulders thus placing an abnormal static strain on spines which are mechanically unfitted for a wide range of movement.

Frequently the symptoms in these patients can be relieved by supporting and resting the involved joints and protecting them from abnormal strain.

On the other hand we occasionally see comparatively young patients with definite hypertrophic arthritis in whom the general health is excellent and the body mechanics are unusually good, and we have learned that we are apt to have considerable difficulty in relieving such patients of their symptoms.

Heberden's Nodes—In addition to the group of patients in whom no mechanical defects can be found to account for the disease there are two very common conditions associated with hypertrophic arthritis which have not yet been explained on a mechanical basis. The most frequent and characteristic of these is the involvement of the terminal interphalangeal joints of the fingers with the development of the enlargements which are commonly called Heberden's nodes and progressive degeneration and deformity of the joints. The development of clubbed fingers in patients with chronic heart disease or supuration in the lungs with insufficient aeration has led some observers to regard the Heberden's nodes as due to either bacterial or metabolic toxemia and others to regard them as the result of an insufficient supply of arterial blood. But anyone who has compared Heberden's nodes with a clubbed finger must have been struck by the obvious difference between the two conditions. The clubbed finger is a uniform hypertrophy of the entire terminal phalanx, bone and soft parts, while the Heberden's node is a production of new bone, cartilage, and fibrous tissue around the base of the phalanx with degeneration of the articular cartilage. Furthermore the Heberden's nodes are frequently not only tender, but may be the site of spontaneous pain and swelling and may be red and resemble an inflammatory lesion either from infection or some toxin or chemical irritant. No stretch of the surgeon's imagination has yet succeeded in attributing the localization of the arthritis in the terminal interphalangeal joints to mechanical causes except in rare instances where they are thought to be occupational.

Subdeltoid Bursitis—A second very characteristic condition which is prone to develop in individuals who are affected with chronic hypertrophic arthritis and which cannot be explained by faulty mechanics is that frequent but poorly understood symptom complex which is variously called subdeltoid bursitis, peri-arthritis of the shoulder, or merely stiff and painful shoulder. Codman has shown that some of these stiff and painful shoulders are due to traumatic rupture of the tendon of the supraspinatus muscle, but this is quite a rare injury, and as a rule we do not know even where the pathology is located, though in the majority of cases we are inclined to incriminate the subdeltoid bursa. In some cases the onset is either sudden or insidious and without known cause and resembles a true infectious or toxic lesion, while in others the symptoms follow a definite injury, particularly a blow or fall upon the shoulder and in still others the cause seems to be occupational. However, in practically no case can the localization of the symptoms in the shoulder be explained by the mechanics of the joint, though it must be admitted that postural enthusiasts are prone to do this when they discover the condition in a round-shouldered patient.

DISCUSSION

From the short review of the literature on experimental arthritis in the early part of this paper it is evident that a condition resembling clinical hypertrophic arthritis can be produced in the joints of experimental animals by various methods which result in injury to the articular cartilage, either di-

rectly or through the production of incongruities in the joint surface with the result that the cartilage is injured by the function of the joint. And it has also been shown that the simple removal of a small portion of the cartilage leads to the same changes.

Likewise the section on traumatic arthritis in man indicates that if the cartilage of a normal joint is subjected to severe injury or to repeated mild trauma from incongruity of the joint surfaces or abnormal function a condition quite similar to hypertrophic arthritis may be expected to develop in the injured joint.

Finally in the section on chronic hypertrophic arthritis the fact has been emphasized that the condition is most often seen in the joints of heavy people who are beyond middle life and that it is especially common in joints which, because of static defects have been subjected to abnormal strains over a period of many years and that the symptoms are often relieved by correction of the static defects.

The three preceding paragraphs suggest that hypertrophic arthritis is simply a worn out joint and that the pathologic picture presented by these joints largely represents the reaction of the joint to an injury and that continued function of the injured joint is necessary for the production of the typical reaction. But a more careful consideration of the disease leads one to the conclusion that such a simple explanation does not suffice and that an answer to many of the questions which confront the student of the disease lies far deeper and must be sought in the individual as a whole rather than in the involved joint.

In other words the joint is a point of lowered resistance where some generalized condition becomes manifest. What this condition is we do not know. Plausible suggestions are low grade infection, vitamin deficiency, some as yet unknown abnormality in the general metabolism, vascular disease of unknown etiology, and absorption of toxic material from the intestine or from foci of infection. It is possible that there are several causes and that one acts in one patient and another in another patient.

In this paper I have not attempted to prove the mechanical functional theory of the disease but have merely tried to emphasize the facts that the pathologic picture of hypertrophic arthritis can be produced by mechanical insults to a joint and that in many cases the symptoms can be relieved by rest and the correction of static defects. We are still seeking the ultimate cause of the disease.

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THE BRITISH RED CROSS SOCIETY'S CLINIC FOR RHEUMATISM

BY A R NELIGAN,* M D, LONDON, ENGLAND

THE Clinic was opened in March of the present year for the treatment of persons suffering from the group of diseases loosely covered by the name "chronic rheumatism." The story of its origin is as follows

Chronic rheumatism has long been known as a cause of serious invalidity in the British Isles. Individual workers on its problems have been numerous, and much good work has been done at the Mineral Water Spas, but no concerted action had been taken as in the case of tuberculosis, cancer, and venereal disease.

In 1923, however, the British Ministry of Health published a report on 'The Incidence of Rheumatic Diseases.' This for the first time opened the eyes of the public and the medical profession to the serious economic and health problems which rheumatism presents.

In 1925 a sub-committee of the International Society of Hydrology was formed to study these problems in different countries, and later a British Sub-Committee was formed. The Approved Societies composed of industrial workers, which provide sick insurance under the National (or State) Health Insurance Acts, by now alive to the great inroads which chronic rheumatism among their members was making on their funds entered keenly into the investigation. Visits were made by laymen and doctors to European countries and the information brought back showed that in several of them, more especially in Germany and Austria, there were well equipped clinics in some of the large industrial centers for treating workers suffering from rheumatism, also that government authorities, industrial, charitable and other organizations had combined in providing facilities at the different spas and health resorts.

In 1927 the British Committee on Rheumatism put forward a scheme for a Demonstration Clinic in London equipped with the different methods of physical treatment, and capable of dealing with several hundred patients a day. The hope was expressed that, if successful, the Clinic would be followed by others. The proposal had a good reception, was well supported by the press, and received the blessing of the Ministry of Health. The question then arose as to who should take it up. Fortunately the British Red Cross Society was found ready to do so. It may be noted here that, since the Great War, the Society, like the United States Red Cross Society, has organized to exercise its beneficent activities in peace as well as in war.

The first question was that of a site for the projected Clinic, it had to be central and with first rate communications. The first essential was not easy to satisfy in overcrowded London except at prohibitive cost and it was eventually decided to convert a very large disused Baptist Chapel for the purpose required. This building put up in 1825 as a panorama was, two years later,

*Medical Superintendent

turned into a Chapel and remained one up until 1922. Just at the southeast corner of Regent's Park its communications are first-rate by main line and underground railway, and numerous omnibus routes.

The cost of converting and equipping the building was estimated at £40,000 and this was quickly collected by public subscription. When the Chapel was first taken over, the baptismal pool was plain to see, and the legend sprung up that the deep pool baths which were eventually made in the bath section of the Clinic, were none other!

Work was begun early in 1929 and was completed about a year later, though improvements are still being made. Many difficulties were met with in convert-

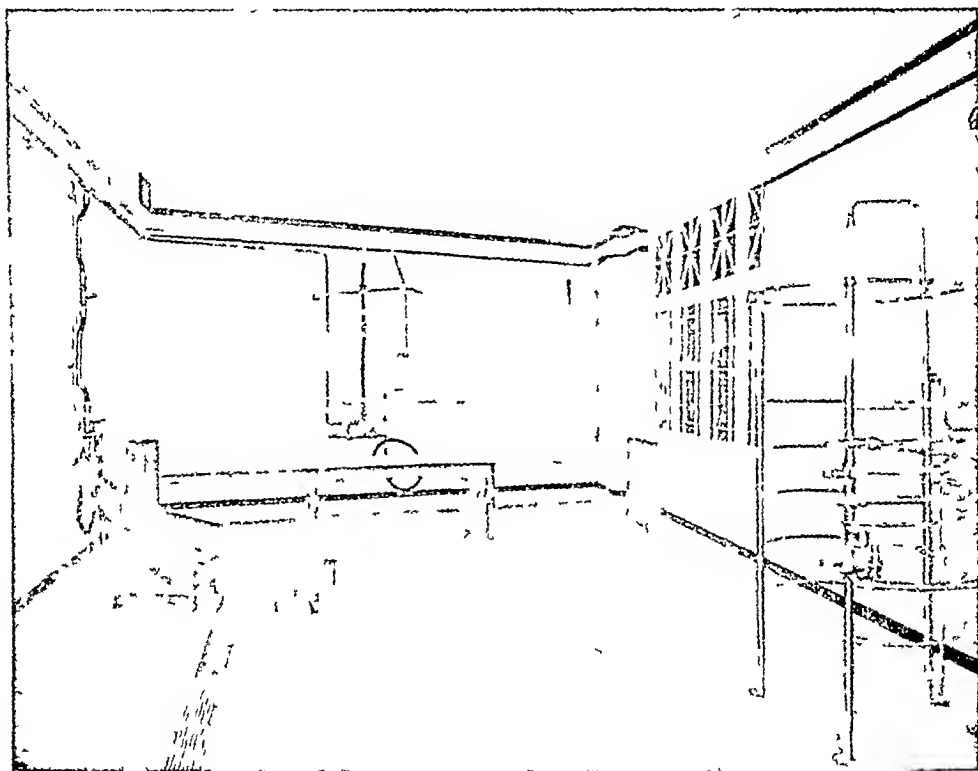


Fig 1—Hydrotherapy section massage douche apparatus and needle bath

ing an old building, with houses on either side, for its outer walls were retained, and they have left their mark on the internal arrangements.

Her Majesty the Queen opened the Clinic formally on February 25 of this year.

Construction and Equipment—The Clinic is now a three floored structure, pierced by a central octagonal shaft, twenty-two feet across, running from the ground floor to a glass dome, for purposes of light and ventilation.

The ground and first floors are given up to patients who can pay only small fees, the second floor is for private patients.

On the ground floor are the Admission Office and Waiting Room and the

Lady Almoner's Offices (she corresponds with the head of the Social Service Department in the United States) The rest of the floor is given up to hydrotherapy

In the center of the octagon mentioned and immediately beneath the glass dome 50 feet above is a circular pool 16 feet in diameter and 4 feet deep This is divided into two equal halves, and one of the halves again divided, so that there are three baths, one large (2416 gallons) which is kept at a temperature of 98° F and two small at 100° and 102° F Each pool has a thermostatic blender for delivering a constant supply of water at a fixed temperature the

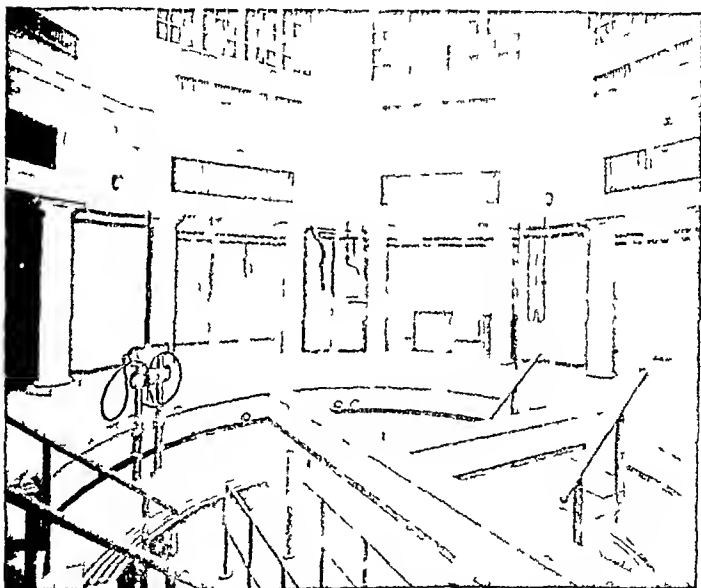


Fig 2—Hydrotherapy section (ground floor) parts of the pool baths

overflow leaves the bath through standing waste pipes 3 feet 6 inches high The baths have teal seats, on which a patient can sit comfortably with his chin above water In the large bath there are parallel bars to enable patients to exercise their lower limbs There is also a thermostatic blender at the side of this bath for underwater douching at a temperature 5° to 10° higher than that of the pool itself, a very useful form of treatment in old muscular and joint conditions The pools are also used for free exercises graduated movements and reeducation of muscles, and manipulation by masseurs who enter the water with the patient

The floor surrounding the pool is paved with ribbed tiles to prevent patients slipping, and is heated

From the central octagon open the dressing boxes (20), two large bays for different water treatments, a hot air room (Turkish Bath) and a vapour room (Russian Bath), with shower nearby, a linen room with hot cupboards, and a room for the masseurs. Reached by a short passage there is a rest room where patients lie on couches, wrapped in a warm sheet and blanket, after treatment, for half an hour or longer. The same corridor leads to the lavatories.

The equipment in the two bays mentioned consists of baths and douches. In the first bay are four shower and foot baths where patients wash before treatment, two reclining baths, both fitted with underwater douches, and one with an aeration equipment, one teak bath for brine baths, one whole body whirlpool bath, and a Scotch douche.

In the second bay is a needle bath, and the equipment for massage douche baths by the Air and Vichy methods, two of each.

The temperature in this bath section is maintained at 70° F. by means of radiators around the walls, high up in the octagon, and steam pipes below the floor. The walls are white tiled, the coping of the pool is blue, and with the gleaming chromium-plated apparatus the bath hall gives a pleasing impression.

On the first floor, reached by lift or stairs are consulting room, examination and booking rooms, two rooms for treatment by massage and electrical methods, one room for carbon arc light treatment, an x-ray room, and room for colonic lavage. The offices of the Medical Superintendent and Sister-in-Charge and a room for the masseuses. There is also a rest hall, and the main linen store. The two main "dry" treatment rooms are equipped with the different varieties of galvanic, faradic and sinusoidal currents, diathermy, luminous and nonluminous radiant heat, ultra-violet light, massage apparatus and a shower bath.

On the second floor private patients are given consultations and receive treatment by practically the same methods as on the ground floor. The only difference is that they have greater privacy. The two deep pool baths are, for instance, individual, and not for several bathers.

On this floor is the clinical laboratory equipped for all the ordinary bacteriologic and biochemical examinations.

In an annex on a higher level than the second floor there are offices for the secretary and accountant, and a tea room for the staff. This leads on to a flat roof, where whatever sun is vouchsafed to London can be enjoyed, with a view of many housetops and the green heights of Hampstead and Highgate in the distance.

On the roof are placed the water storage tanks, seven in all, of one thousand gallons each.

The water used for treatment is ordinary London water which has been softened on the premises. The heating plant is by two vertical steam boilers with two 300 gallon calorifiers.

The electricity supply from the main is direct at 240 volts. This is stepped down for treatment purposes to 100 volts, and also converted into alternating current at 100 volts, in the switch room.

It will be noted that there is no mention of beds, the Clinic is designed for the treatment of ambulant patients only.

Patients—While the Clinic has been founded to help the industrial worker in particular, all classes of patients are accepted, provided they bring with them from their medical attendants a certificate to say that they are suffering from rheumatism. All are expected to pay towards the cost of their treatment, for the Clinic has no endowment fund and it is hoped that it will become self supporting.

As regards the poorer class of patient, they are divided into two groups.

1 Noninsured persons, their fees are arranged by the Lady Almoner to suit their circumstances. A certain number of free cases naturally are accepted.

2 Insured persons that is to say contributors to the National Health (or State) Insurance Scheme. They are in most cases members of "Approved Societies" and these have received authority from the Ministry of Health to pay out of their funds, a capitation fee of three shillings (75 cents) per treatment, or 25 shillings for a course of 9 treatments. This sum covers consultations but not drugs (provided under the Act). X-ray examination is counted as a treatment.

These are trial figures which may be adjusted in the future. The Clinic is the only institution recognized by the Ministry for the purpose and the payment is not yet authorized by law as in the case of the "additional benefits" for specialist treatment of the teeth or eyes. It is expected, however, that if the Clinic is a success the treatment of rheumatism at institutions approved by the Ministry will be made an additional medical benefit under the Insurance Act.

Here again the patient is accepted for treatment only on the recommendation of the insurance general practitioner on whose "panel" he is.

A number of charitable organizations and Friendly Societies are seeking and obtaining the same arrangements for their members.

Private patients pay roughly ten dollars for their first and five for subsequent consultations. These fees go to the Visiting Physicians. The charge for each attendance for treatment is roughly two and a half dollars paid to the Society.

All patients must be seen and all treatment prescribed by a member of the Staff. Consultations and treatment are given by appointment only.

Treatment is given to the two sexes on alternate days between the hours of 9.30 A.M. and 8.30 P.M. A special feature is being made of evening consultations and treatment out of hours for day workers. This is much appreciated and would seem to be the only way of getting early and slight cases to pay attention to their condition.

The Medical Staff of the Clinic consists of five visiting physicians who are specialists in treatment by physical methods, or general physicians interested in the rheumatic group of diseases. Each attends twice a week to see patients. The Surgical Staff of the Clinic is supplied by the Royal National Orthopedic Hospital which is hard by. There is a pathologist and a radiologist. Other specialists are in course of being appointed.

The Nursing Staff consists first of the Sister in Charge, who is a trained nurse as well as a qualified masseuse. She has two assistants and a staff of 4 masseuses for the dry treatment rooms and 16 masseuses for the bath section.

some of the latter are students in hydrotherapy. There are eight bath masseurs, some again of whom are students. All treatment assistants and students have the certificates of the Chartered Society of Massage and Medical Gymnastics, which demands a two years training in anatomy, physiology, massage, remedial exercises, and medical electricity. The Society has the intention of introducing a certificate in hydrotherapy, and the Clinic will be a training school for it.

On account of the long hours during which the Clinic is open, many members of the treatment, social service and administrative staffs have to be duplicated, and this adds greatly to the running costs. It is estimated that these will amount to some £15,000 a year.

Consultations are held morning and afternoon and three times a week in the evening between 5 P.M. and 8 P.M. Treatment is carried on daily from 10 A.M. to 1 P.M. and from 2 P.M. to 8 P.M. or later. The provision of evening consultations and treatment after work hours is held to be essential to any scheme of dealing with the problem of industrial rheumatism.

The *responsibilities* accepted by the Clinic are as follows:

1 Diagnosis

Selection of cases for spa hospital and other outside treatment

2 Treatment

3 Follow up and welfare work

4 Education of

Medical students

Postgraduates

Treatment assistants

Patients (prevention)

5 Research into the causes, prevention, and treatment of rheumatism

The *nature of the conditions* accepted for treatment is as follows:

1 Chronic rheumatic diseases

(a) Articular, i.e., chronic (nontuberculous) arthritis, including spondylitis and gouty arthritis

(b) Nonarticular, i.e., lumbago and other forms of muscular rheumatism, sciatica, fibrositis, etc., etc

2 Muscular weakness or paralysis, neuritis, neuralgia

3 Sequelae of injury to or inflammation of joints, muscles, tendons, bones and nerves

Types of Rheumatism—Patients with rheumatism may be, from the point of view of treatment, divided into two great groups:

1 Those with definitely chronic disease, more or less fixed deformities and more or less pain

2 Those in the early stages, the prearthritic, early rheumatoid or infective arthritis, early muscle and nerve rheumatism, and so on

The latter class is well worth much time and care, continuous for months or years it may be, it is the class which best rewards effort, and should, where bed treatment is not necessary, be the special object of a clinic such as this.

For the first class little can be done in the way of permanent, but a great deal by repeated treatments in the way of temporary relief. Unfortunately this class is very numerous and very painful, and absorbs much energy and

time It forms the bulk of our patients so far, and it presents this problem, well foreseen, which every clinic of this sort must face how to prevent the clogging of the machinery of treatment by large numbers of advanced cases

Another point which comes out is that women patients greatly exceed men This is a common feature of treatment centers for rheumatism The difference will be less marked as the Approved Societies take fuller advantage of the scheme, for the majority of insured workers are men

Taking our statistics as far as they go, the cases fall roughly into three groups the rheumatic, the medical and the surgical The existence of the two latter groups shows that useful work is being done in diagnosis, in them have been found cases of central nervous lesions, appendicitis, metastatic newgrowth, orthopedic conditions, etc

An analysis of rheumatic patients seen, shows that they were suffering from the following conditions, and in the proportions given The numbers available are small, but they will serve to give some idea of the run of the clinical work Decimal points have been omitted

The *Nonarticular conditions* form 28 per cent of the whole, distributed in the following proportions

	MALES PER CENT	FEMALES PER CENT	TOTAL PER CENT
Fibrositis	8	25	33
Lumbago	7	3	10
Lumbosacral conditions	7	3	10
Myalgia	7		7
Neuritis		10	10
Sciatica	22	3	25
Vague rheumatic symptoms		10	10

Arthritis accounts for 72 per cent of the whole, distributed in the following proportions

	MALES PER CENT	FEMALES PER CENT	TOTAL PER CENT
Climacteric		5	5
Gouty	1	2	3
Infective	10	6	16
Osteoarthritis	20	10	30
Prearthritic conditions	1	2	3
Rheumatoid arthritis		20	20
Spondylitis		1	1
Unclassified	1	10	11

Classification of Arthritis—The term rheumatoid arthritis is being confined by the Medical Staff to cases of the atrophic type in women Osteoarthritis corresponds to hyper trophic arthritis

Early Results—First impressions point to the Clinic filling a real need The small nucleus treatment staff with which a start was made has had to be progressively increased, and additional space and apparatus have had to be brought into use, to meet growing demands Still further facilities are shortly to be provided The applications for treatment are numerous and there is a long waiting list of women patients In March (twenty days) there were 680 attendances for treatment, in April 1844, in May 4487 The average daily attendance has been 145 The following brief analysis gives an indication of the

numbers and kinds of treatments administered Series treatments are commonly prescribed, two or three at an attendance

Massage and electrical treatments 2195 in the month of May

Diathermy	745
Massage and Remedial Exercises	527
Infra Red Rays	250 (general)
Ultra Violet Rays	192 (general)
Schnee Bath	169
Radiant Heat	108
Galvanism	88
Faradism	87
Kromayer Lamp	21
High Frequency	8

Under "diathermy" are included general diathermy and pelvic diathermy (cervix and prostate) With the Schnee bath, suiging sinusoidal and galvanic currents are given, rheumatoid conditions of the hands and feet and muscular wasting and brachial neuralgia have been the common indications The treatment of certain types of infected tonsils and local rheumatic lesions, with the water cooled Kromayer lamp, is being tested

BATH TREATMENTS 5202 IN THE MONTH OF MAY

<i>Reclining Baths</i>	
with aeration	180
plain	64
with underwater douche	44
medicated	36
	324
<i>Pool Baths</i>	
with underwater douche	734
with manipulation	28
with free exercises	14
	776
Massage douche—Air method	636
Vichy method	351
Needle bath	1022
Scotch douche	315
Hot air room (130° 140°)	932
Vapor room (100° 104°)	231
Packs	73
Whirlpool bath	87
Colonic lavage	165
Massage after bath treatment	290

The reclining bath with aeration is useful as a tonic treatment for patients unable to stand the more vigorous forms of hydrotherapy, and as a method of "skin training" The medicated bath most used is the bime bath, sulphur and alkaline baths are occasionally ordered The value of the pool or deep immersion bath, generally at 98° or 100°, and with underwater douching, in arthritis and fibrositis is well known Air massage douche baths are prescribed especially for local conditions, Vichy for more general treatment They are preceded by five to ten minutes in the hot air room in order to stimulate the skin, and followed by a needle bath cooled to 80° or 70° F, hence the high numbers for these two forms of treatment For local treatment by packs,

fuller's earth is used. Colonic lavage is given with a simple irrigator and an alkaline isotonic solution, to which potassium permanganate is occasionally added.

It will be seen from this brief resume that, for the moment, treatment is centering round the hydrotherapeutic measures, except for diathermy and dry massage, both of which are much prescribed. The "heating" treatments, too, whether dry or wet, radiant or non-radiant are also high in the list.

As to the actual results of treatment it is obviously too early yet to say much. It is quite certain that a large number of sufferers have been given relief as regards pain and disability for the time being.

It is estimated that when the additional accommodation and apparatus in view have been provided eventually more than 200 patients can be treated in the large bath section daily, 120 in the massage and electrical rooms, and 70 on the private floor.

Research—If there is little to say on this subject, it must be remembered that the starting and organization of treatment in a new type of institution has been no light task. For various reasons, too, plans have had to be curtailed, but already data are being collected on the flora of the throat and certain types of new treatment or modifications of old are being tested. It is intended that research into the causes of rheumatism shall receive full attention.

Rheumatism as an Industrial Disease—The opening of the Red Cross Clinic marks, as has been said, the first step of a movement to provide on a large scale in England treatment for rheumatism, especially among industrial workers. Our own experience is too short to add anything to what is known already on the subject of industrial rheumatism but it will not be inappropriate to accede to the editor's request and add a few lines on the subject, to what has already been said in the first part of this account. The facts and figures which follow are nearly all taken from the reports of the British Ministry of Health.*

The proportion of patients in industrial practices in England who suffer from rheumatism is roughly 5 per cent. (D. K. Brundage of Pittsburgh U.S.A. made it 6.4 per cent among steel workers.) An estimate of insured workers in this country made in 1927 was however, no less than 9.8 per cent.

The proportion of lost working days due to rheumatism (rheumatic fever included) is no less than one sixth of the total caused by all diseases. In 1927 some 5,000,000 weeks were lost owing to this cause.

The cost of this huge mass of invalidity in sick pay was £5,000,000 and in addition £12,000,000 was lost in wages. More than half of this great total of rheumatic sick absence for both sexes is due to chronic joint diseases principally rheumatoid arthritis, and osteoarthritis in nearly equal shares. The proportion due to nonarticular manifestations was one third in men and one-quarter in women.

The average age of insured females is less than that of insured males the following figures are, therefore, not strictly comparable. They show the expected attack rate per 1000 of the two sexes in a year.

	MALES	FEMALES
Arthritis—rheumatoid	1	3
ostearthritis	3	2
gout	3	0 1
unclassifiable	1	
Muscular rheumatism	6	7
Lumbago	10	3
Seritica	3	1
		(or brachial neuritis)

As regards occupational incidence, the Ministry's inquiry brought out the liability of metal workers to rheumatic disease, it is "nearly 80 per cent in excess of expectation" In steel works the puddlers are especially liable, alternating heavy work, exposure to heat and chilling of the body surface must have much to do with this The tin-plate men on the other hand, working more or less continuously, do not suffer excessively The effect of exposure to weather is shown in the liability of the London tramcar driver to rheumatism, especially if he has no protection Exposure to heat in industry is not a predisposing cause, but if accompanied by damp, it is These facts call to mind the importance of the skin functions in rheumatic subjects It is, therefore, interesting to know that the provision of baths at coal mines has begun In another direction the provision of dental treatment under the National Health Insurance Act should have an effect on the incidence of rheumatism among the working classes

The figures given of working days lost and the cost to the community of rheumatism demand its closest attention, especially when it is, as in England, an industrial one But they apply to the insured population alone, say 16,000,000 souls, and, further, they utterly fail to convey any adequate impression of the misery, the inefficiency, and the mental strain, for which rheumatism is responsible

Surely chronic rheumatism is one of the major problems of medicine and perhaps the most difficult one of all

PFTO PLACE, MARLBORNE ROAD, N W 1

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A KILDUFFE MD ABSTRACT EDITOR

ALLERGY *New Method of Administering Pollen Extract for the Purpose of Preventing Reactions* Duke W W J A M A 94 767, 1930

The method of administering pollen described here is designed to control the rate of dissemination from the site of inoculation and depends on four important steps

1 The placing of a rubber tourniquet around the arm above and just prior to the injection of the pollen and its frequent removal and replacement for at least five minutes after the injection

2 The admixing of epinephrine and ephedrine with the pollen solution in such dosage as to slow down its rate of dissemination from the site of injection

3 The reducing of all doses to a given volume by a admixture of physiologic solution of sodium chloride

4 The introduction of 0.01 cc of the pollen mixture subcuticularly preliminary to the injection of the entire mixture The appearance of a tiny blanched area will indicate that the needle is not in a vein and the entire mixture if injected will go into the subcutaneous tissues

TUBERCULOSIS *Method for Rapid Demonstration of Tubercle Bacilli* Pfannenstiel W Deutsche Med Wchnschr 55 2130 1929

Pfannenstiel described a method by means of which it is possible to demonstrate the presence of tubercle bacilli in a comparatively short time He inoculates guinea pigs with material from patients The injection is made into the animal's popliteal lymph nodes Whenever the material contains tubercle bacilli the lymph nodes become enlarged and hardened in the course of the second week following the inoculation Then they are extirpated and crush preparations are made These are stained and examined under the microscope The entire process requires only about two weeks The author performed this test on 150 animals

MOSQUITOES *An Improved Deterrent* Dover C Indian J Med Res 17 961 1930

Citronella oil (Burgoyne's)	½ ounce
Spirits of camphor	¼ ounce
Cedar wood oil	¼ ounce
White petroleum jelly (B.P.)	2 ounces

The petroleum jelly should be melted and the other constituents then added the mixture being well stirred Bottle (a 3 ounce wide-mouthed jar is a convenient size) and cool rapidly preferably by placing the bottle (which should be kept closed) in a basin of cold water or in a refrigerator

The formula forms a firm, whitish nonstaining cream of pleasant odor which in addition to its properties as a mosquito deterrent is soothing antiseptic and beneficial to the skin (petroleum jelly it will be remembered is the base of most face creams) One application usually lasts for a whole night and only a small quantity need be used on each occasion To avoid using it on the face in the evenings the cream has been employed by some as a brillantine for the hair as it was found that for a time this keeps mosquitoes away almost as successfully as if the whole face were smeared with it

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EDITORIALS

A Symposium on Arthritis

IN THE cycle of medical interest certain diseases or disease groups come rather periodically to the fore. This is equally true with popular interest in medical subjects. Following the "Swat the Fly" campaign, the "Don't Spit" campaign, and the "Sanitary Drinking Cup" campaign, all of which developed along with the growth and activities of the National Tuberculosis Association, interest next centered in eugenics, with consequent development of eugenic societies and race betterment associations. Then came the popular interest in cancer.

In both medical and popular circles, flare-ups of interest in certain subjects depend, naturally, on various causes. A new discovery holds out hope of an early Utopia, an apostle sways the multitude in the direction of his own interest and enthusiasm, a disease such as influenza, almost forgotten, claims our interest because of its returning prevalence.

Rather in contrast to this, there are problems in medicine which are with us at all times—problems toward the solution of which many of our most

capable workers are bending their best endeavors, concerning which it seems well to direct general attention from time to time in order that we may be cognizant of what progress is being made and of how diverse or even conflicting observations are being, or must be, correlated or reconciled.

The symposium idea in publications, while in no way new in this country, has been applied rather more successfully among certain of the more prominent continental journals.

A symposium, to be of the greatest value, should not be a series of discussions or a sequence of etiology, pathology, symptomatology, diagnosis, and therapy, contributed by reviewers whose qualifications rarely justify their being termed authorities. Rather should it be a series of presentations by leading authorities in their fields. Nor need the individual contributions be closely correlated. The reader will have no difficulty in providing his own correlations. Too often attempts to fit one's contribution into the pattern of a mosaic lessens the forcefulness of the contribution as an independent unit.

Some clinical journals have rather specialized on symposia, but as a rule they have been of a group which lacked sufficient prestige to interest the leaders in the fields in contributing. There can be no doubt that the symposium idea is an excellent one provided the contributions are from men of eminence. Symposia at medical conferences, the Symposium on Nephritis just terminated at the University of Minnesota, volumes such as those recently edited by Jordan and Falk by McClung, by Rivers, and by Cowdry bear evidence of this.

It is for reasons such as these that the editor is glad to offer to the readers of the JOURNAL a symposium which is of general interest, both in its clinical and its laboratory aspects. If the subscribers should judge it a success and should desire additional symposia on topics of interest we will be glad to issue others occasionally. The new and original contributions herein were made on invitation and the editors express their deep appreciation to the essayists for their interest and cooperation.

One who reads the pages of this number* will recognize at once the tremendous economic importance of the subject under discussion and its diverse aspects. To those who have made no intensive study of the subject, the many facets of the investigations will be stimulating. The variety of etiologic factors (several different organisms are suggested), colonic absorption, local nutritional disturbances, endocrine imbalance, dietary factors, climate, even the possibility of protozoan infestation, all come in for discussion.

Dr. Ely has said: "One of the most popular methods of treating patients with arthritis is to send them to some one else." This, regrettably, is too often true, and if continued from physician to physician in an endless chain, deprives the victim of the disease of those perfectly definite and helpful therapeutic aids which have so far been established and are his by right.

It is in the hope that these essays will stimulate the clinician to a broader and more intensive therapeutic outlook and a greater interest in the welfare of his arthritic patient and the pathologist to renewed efforts to solve the mysteries of the disease that this symposium is offered to our readers.

—W T V

* It was originally planned to have but one number devoted to a symposium on arthritis but on account of the length and excellence of the material we have decided to issue it in two numbers.

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News Item

The following officers were elected at the June meeting

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SYMPOSIUM ON ARTHRITIS—Continued

A MODERN CONCEPTION OF ARTHRITIS

BY RUSSELL L. CECIL, M.D., NEW YORK CITY

ARTHRITIS, long one of the mysteries of medicine, is beginning to yield some of its secrets to modern investigators. The present day student of this disease makes a primary and fundamental distinction between arthritis due to infection and arthritis dependent on other etiologic factors. Nearly all pathogenic bacteria can, under proper conditions, invade the joint and excite inflammation in it. This is particularly true of the common pathogens of the oral cavity. The bacteria most prevalent in mouth secretions are the staphylococcus, *Streptococcus hemolyticus*, *Streptococcus anhemolyticus*, *Streptococcus viridans*, *Pneumococcus*, and *Bacillus influenzae*. All of these bacteria, including the Pfeiffer bacillus, can excite an arthritis. Strangely enough the *Streptococcus viridans*, which is the commonest of the mouth inhabitants and the organism most frequently found in tonsils and root abscesses, is the one pathogen which some investigators are most loath to admit as an exciting agent of arthritis. Yet a priori it should be the most frequent excitant of the disease.

Infection is responsible for two thirds to three fourths of all cases of arthritis, but there remains a large group of cases which are apparently noninfectious in origin. By far the most prevalent in the noninfectious group is the so called hypertrophic or degenerative arthritis. All the pathologic and bacteriologic evidence indicates that hypertrophic arthritis is not referable to bacteria, but to the phenomena which accompany senescence.

ACUTE ARTHRITIS

Acute arthritis is usually a complication secondary to bacteremia. Why is it, however, that many patients with bacteremia escape arthritis, while others contract the disease? Two factors probably come into play: first, trauma, and second, hypersensitiveness of the joint tissue. It is a well known fact that an injured joint is prone to infection, constituting by reason of the injury, a

point of lowered resistance. But modern studies, particularly in the domain of rheumatic fever, indicate that bacterial allergy is also instrumental in some cases. Even in rheumatic fever, a disease which is so frequently associated with swollen joints, cases are often seen, especially in children, where the joint involvement is insignificant, the disease manifesting itself in the heart, pericardium, pleura, or brain.

In recent studies conducted in the laboratories of Bellevue Hospital,¹ streptococci usually of the green type have been recovered in a high percentage of cases from both the blood and the joints of patients with rheumatic fever. From this we infer that though bacterial allergy may play an important part in sensitizing the joint tissue, the actual development of rheumatic arthritis in the joint is an infectious process due primarily to the presence of streptococci in the joint tissue. In other words the pathogenesis of rheumatic fever differs in no essential respect from that of gonococcal arthritis. In both diseases the sequence of events is

- 1 Focal infection
- 2 Bacteremia
- 3 Localization of bacteria in joint, arthritis

CHRONIC INFECTIOUS ARTHRITIS

Investigators of rheumatic conditions have long been impressed with the close similarity between rheumatic fever and chronic infectious arthritis. Both tend to occur in young people, though rheumatic fever is more common in children, and infectious arthritis in young adults. Both diseases appear to be related to foci of infection. Both are characterized by swollen, painful joints. Both may present all the appearance of an acute infection in the early stages, so much so that it is often impossible to differentiate them clinically, both may go on to a chronic form, and about 5 per cent of patients with rheumatic fever progress into a form of chronic arthritis indistinguishable from rheumatoid arthritis. Both diseases may at times involve the muscles and tendons as well as the joints, and both diseases are sometimes complicated by subcutaneous nodules.

A final and quite important point of similarity between rheumatic fever and infectious arthritis has been brought out by the studies in our laboratory (Ceel Nicholls, and Stainsby²). We have found that in chronic infectious arthritis, as well as in rheumatic fever, cultures from the blood and joints will yield streptococci in a high percentage of cases. With rare exceptions the streptococci recovered from arthritic patients differ culturally and biologically from streptococci isolated from patients with rheumatic fever. In the former disease an intermediary type of streptococcus, possessing both hemolytic and green-producing properties is usually found. In rheumatic fever green streptococci are isolated in the great majority of cases, though occasionally an indifferent streptococcus, of the type described by Small³ and by Birkhaug,⁴ is recovered. Recent studies on the agglutinations are bringing out some interesting facts. Patients with typical chronic infectious arthritis show high agglutinins for the typical arthritic strains in 94 per cent of cases. In rheumatic fever the agglutination reactions are not so strong, but nevertheless are quite definite.

The green streptococci recovered from rheumatic fever patients appear to fall into definite biologic groups analogous to the pneumococcus groups

The life history of rheumatoid arthritis is similar in all respects to that of rheumatic fever in presenting the same chain of events, namely, focal infection, bacteremia, and metastatic infection of the joints. In addition to the joint lesions, bacteria sometimes localize in the muscle or tendon sheaths and occasionally in the subcutaneous tissue, with the production of subcutaneous nodules. Complications, such as pleurisy and pericarditis, are not so common in infectious arthritis as in rheumatic fever though they do occur. McCrae has reported a series of cases of rheumatoid arthritis manifesting pleural or pericardial symptoms. If the streptococcal theory is correct, it is difficult to explain why endocarditis and Aschoff bodies occur with such high frequency in rheumatic fever, whereas in infectious arthritis these lesions are practically never seen. This difference can hardly be dependent on the age of the patient as children with rheumatoid arthritis (Still's disease) rarely show valvular lesions. The difference must therefore depend upon something in the streptococci themselves.

In order to control the cultures on patients with rheumatic fever and in infectious arthritis, blood and joint cultures have been made on normal individuals and patients with conditions other than rheumatism or arthritis. We have never succeeded in recovering a streptococcus from the blood or the joint of a perfectly healthy individual. In four or five instances green streptococci have been isolated from blood cultures of patients with some acute respiratory infection or with some chronic focus of infection such as pansinusitis or chronic tonsillitis. Such findings do not appear to us to militate against acceptance of the streptococcus as a cause of rheumatism and arthritis. It is a well known fact that both of these diseases usually develop after some acute respiratory infection or in patients who harbor some chronic focus of infection about the throat sinuses, or teeth. It is not surprising therefore, that streptococci should occasionally be encountered in the blood streams of patients with these infections.

ANIMAL EXPERIMENTS

Final proof that the streptococcus causes rheumatic fever and infectious arthritis will depend upon the success with which these diseases are reproduced by the streptococci in animals. The animal which has been employed almost exclusively up to the present time is the rabbit. Poynton and Paine,⁶ Rosenow,⁷ Clawson,⁸ and others have reproduced lesions in rabbits with the streptococci recovered from patients with rheumatic fever, and they claim that lesions so produced are practically identical with those which occur in man. There has been some disagreement on this point, however, and pathologists have some difficulty in deciding just what an Aschoff body is. Reproduction of chronic infectious arthritis in rabbits has been a little more successful. Numerous workers, including Rosenow,⁷ Haden,⁹ Burbank,¹⁰ and ourselves have produced in rabbits chronic joint lesions which bear every resemblance to the human lesions. In our own studies some of the rabbits have developed true deformities and sections taken from the joints are indistinguishable under the microscope from sections taken from the human arthritic joint. Furthermore, cultures

from the blood and joints of the arthritic rabbit usually yield a streptococcus which is culturally and biologically identical with the strain injected

The recovery of streptococci from a high percentage of patients with rheumatic fever and infectious arthritis, and the reproduction with these streptococci of lesions in rabbits so similar to those in man, make it difficult to resist the conclusion that both rheumatic fever and infectious arthritis are streptococcal infections. The proof is not yet final, but certainly a great deal of evidence has accumulated to support the streptococcal theory

HYPERTROPHIC ARTHRITIS

As already indicated, hypertrophic arthritis does not present the picture of an infectious disease. Clinically the patients are without the fever, the leucocytosis and the secondary anemia which those with rheumatic fever or infectious arthritis so frequently present. The patient with hypertrophic arthritis is nearly always middle-aged or elderly, and one gets the impression that the joint lesions are simply another phase of senescence.

The pathologic changes in the hypertrophic joint are not those which characterize infection. In infectious arthritis granulation tissue spreads over the articular surfaces and may eventually fuse them together. In hypertrophic arthritis there is no granulation tissue or other evidence of an inflammatory reaction. The cartilage is worn down and fibrillated and the underlying bone is increased in density. Around the margins of the joint the bone is hypertrophied and small bony spurs grow out. Fusion of the articular surfaces does not occur.

The bacteriologic and serologic findings in hypertrophic arthritis give no evidence of an infectious process. In our experience cultures from the blood and joint have been sterile without any exception, and agglutination tests carried out with the patient's blood have never given positive reactions with any of the streptococcal strains. Studies conducted by Pemberton¹¹ and more recently by Hench¹² indicate that hypertrophic arthritis results from inadequate blood supply, the latter in turn being due to an endarteritis of the small vessels. Improper nutrition of the joint results in a lowering of its resistance to ordinary wear and tear, and when the strain on the joint is excessive, such as exists in the knees of an overweight patient or in the shoulders, fingers and back of certain laborers, hypertrophic arthritis develops. It appears, therefore, that endarteritis is a predisposing cause and trauma the exciting cause of hypertrophic arthritis.

SUMMARY

So far, this paper has been confined largely to a discussion of the exciting agents of arthritis. Before closing, perhaps something should be said about the various predisposing influences which are at work in these diseases. In rheumatic fever the most important predisposing factor is environment. Rheumatic fever is a rare disease among the well-to-do. It is extremely common among the children of the poor. This increase of susceptibility in the poor is probably a combination of improper diet and exposure to cold. The well-to-do are better fed and better clothed and escape infection. Probably another pre-

disposing factor is exposure to a carrier of the rheumatic fever virus St Lawrence¹³ has pointed out the prevalence of rheumatic fever in two or more children of the same family This may be heredity, but contagion may also be an important factor

In both rheumatic fever and infectious arthritis focal infection is of vital significance Statistics show that rheumatic fever cannot be prevented by the removal of all foci of infection, but the incidence is considerably reduced

Other factors are influential in the etiology of infectious arthritis Among them, nervous or emotional shock should be stressed It is surprising what a large percentage of these patients give a history of overwork or some emotional disturbance just prior to the onset of arthritis Other factors such as trauma and exposure to cold and dampness sometimes provoke the first attack I have seen a number of butchers who claim to have contracted arthritis from too many trips to the refrigerator

In hypertrophic arthritis overweight and advancing years are the two great predisposing agents Heredity also plays an important part Nearly every patient with Heberden's nodes will give a history of a mother or father who was afflicted with the same condition

This discussion has been nothing more than a cursory review of a complex subject but perhaps enough has been said to indicate that some of the mysteries of arthritis are on the way to solution and that with a few more years intensive research along bacteriologic and physiologic lines the medical profession will have a fairly concise idea as to the true nature of rheumatism and arthritis

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CLINICAL OBSERVATIONS IN CHRONIC ARTHRITIS

BY LORING T. SWAIM, M.D., BOSTON

THIS review of chronic arthritis is based on seventeen years' observation of patients suffering with joint disease. Certain clinical aspects of these patients have impressed themselves as constantly present and of increasing significance. Since we do not yet know with certainty any one precipitating etiologic factor in the production of arthritis, all data are of importance.

It is generally accepted from a pathologic standpoint that there are two widely divergent types of nontuberculous joint disease, the atrophic and the hypertrophic arthritis. The first is a proliferative reaction¹ about the joint, with general bone atrophy, and the second a degenerative disease of the bony structures, with secondary soft tissue changes. Many place the infectious arthritis in the same group with the atrophic. There are, however, numerous clinical factors in the atrophic picture which are not explained by the bacterial theory alone. Having had the opportunity to study cases over years rather than months, certain differences stand out with increasing clearness as the years go by. For these reasons,² the three types of arthritis are used as a means of accurate observation and recording of data.

It has been our experience that most cases of infectious arthritis,³ even when the disease is severe and overwhelming, do not progress, even in years, into a typical atrophic case, and have very few of the characteristic signs of the latter disease. The only point of resemblance may be the joint manifestations. The constitutional signs and symptoms are not the same. For example, ankylosis occurs in both infectious and atrophic arthritis, but general bone atrophy does not follow in the infectious type, even after years of ill health and invalidism. It does from the first in the atrophic.

There are cases of arthritis undoubtedly due to bacterial infection, where some focus of infection either starts a blood infection and locates in the joint, or makes the joint hypersensitive to its toxins, and therefore an allergic joint is produced. Those foci are in tonsils, teeth, sinuses, intestines, and urinary tract. These cases respond to removal of the infected focus (if it is removed early), and the joints have not become too sensitive to the foreign poisons. They usually are better for the removal of the source of toxemia, and are frequently still further improved by careful use of vaccines. Foci of infection are better removed whenever possible, if by so doing the operation in itself is not more than the patient can stand in his depleted, oversensitive condition. Otherwise he has a better chance to recover in the presence of the focus by general supportive treatment until his immunity, or resistance, has been brought up to a point where removal is safe.

An example of this is a woman who came to the hospital three years ago with a general arthritis, from which she had been incapacitated for a year.

and a half The x ray study showed no bone atrophy Clinically, there was little muscle atrophy a few joints were very nearly destroyed, with thin cartilage, much soft tissue proliferation, and considerable loss of weight The laboratory study was as follows

Hemoglobin, 70 per cent, RBC, 4,500,000, WBC, 16,200 Smear polymorphonuclears 50 lymphocytes, 30, large mononuclear cells, 6 eosinophiles, 4, tr, 2

Galactose Tolerance 30 gm ++, 20 gm perceptible trace, 15 gm + 10 gm light trace, 7 gm 0

Blood Chemistry Sugar, 87 133 138 128, Nonprotein Nitrogen, 35.1 Ca, 11.2 Ph, 11.6

Basal metabolism, -16, -23 -26 Vital capacity, 2600 On discharge metabolism still -20

The tonsils were hadly infected Because of her general condition they were not removed at first She was put to bed, given an ample, well balanced diet, slightly low in carbohydrates She had hydrotherapy massage, and corrective body exercises to improve her posture She was built up gradually until her joint symptoms of pain, heat, and tenderness had subsided After four months she was up and about with no active arthritis She had developed her own resistance Then her tonsils were removed, and she has had no recurrence of symptoms, objectively or subjectively, since She has gone back to a normal, unrestricted life Some joints are still damaged, but are usable and increasingly so This woman, with a demonstrable focus, depletion, and crippling after a year and a half of active arthritis, showed no tendency to the bone changes constantly present in the atrophic patient

Infectious arthritis does not have any special age, sex, or type proclivities It is not a respecter of persons, which cannot be said of either the atrophic or hypertrophic arthritis

The patient with hypertrophic arthritis never has much trouble from his joints until late in life, and then only when he acutely injures some joint, or when the degenerative changes have reached a point where motion becomes painful and restricted The degree of joint change seems to depend principally on the amount of daily trauma the joint receives, and is usually found chiefly in the large working joints such as the low back, hips knees and cervical spine Other joints are affected, but it is usually the weight bearing ones which receive daily strain, often legitimate but quite frequently from unnecessary postural misuse

It puts in its appearance late in life, and the degenerative changes are probably largely due to trauma and the inevitable physiologic changes due to advancing years It is rarely found except in the heavy type of person, and is twice as common in men as in women This type of arthritis is primarily an orthopedic problem and is not difficult if the traumatic idea is remembered unless, of course, the degeneration has so changed the joint that all motion is painful The principle of treatment is to stop the daily injury by rebalancing the body after the joints have been rested This is a question of postural correction and changing the weight bearing of the joints from their strained angles with back supports plates, elastic knee supports, and especially training

Much can be done to make the effects of age less apparent by endocrines,⁶ diet, and increased elimination, much as one does for the arteriosclerotic, as many of these cases show arterial changes as well

The most interesting and difficult cases to treat are those with atrophic arthritis,⁷ as they present a complicated physiologic condition. Atrophic arthritis has a distinct hereditary tendency. It is three times as common in woman as in men, and is a disease of early middle life. But almost invariably it appears in the slender, light-boned woman, the congenital visceroptotic type. It is almost never found in the heavy-boned people. These facts have made the controversy between infection and constitutional tendencies an ever-present one. Clinically, the general bone atrophy, the exaggerated muscle atrophy, and the extreme lack of tone, occur early in the disease. There is much data which suggest a constitutional background for the disease. Just which one of many apparent causes precipitates the final break in compensation is not yet certain, but such things as exhaustion, chill, infection, pregnancy, nervous shock, the results of postural defects, all seem to be causes in different cases.

The outstanding clinical facts in these cases are their vasomotor instability, their sympathetic debility, and the depletion of their metabolic vitality, as manifested by subnormal body temperature, their abnormally cold extremities, often 20° below body temperature, their low systolic blood pressure, often below 100°, and a low pulse pressure. In a series of 300 cases of arthritis,^{6, 8, 14} the basal metabolic rate was below -10 in 60 per cent, and below 0 in 83 per cent. There is usually a chronic, secondary anemia. X-ray studies of the intestines reveal an atonic large bowel with diminished haustral markings, suggesting a low grade colitis.

The atrophic arthritic is very unstable in his superficial circulation with rapid vasomotor changes, manifested by cold, blue hands and feet,^{5, 7, 9} and mottling of the skin. He is, therefore, susceptible to cold, changes in weather, and barometric changes often cause acute suffering.

It is interesting to note that these symptoms are frequently present in varying degrees long before the joints are noticed, and are exaggerated as time goes on and the joint symptoms begin. They are warnings of disturbances which precede atrophic arthritis, which is only waiting for the chance cause. Millard Smith recently has suggested that this is part of the disease¹⁰ (arthritis without joint involvement). We feel that these disturbances are forerunners of the inevitable atrophic changes unless they can be corrected.

In a recent series of papers, Kuhns and I have published our convictions^{11, 12, 13} that the deformities of arthritis are largely unnecessary, except in exceptionally severe cases. The joint damage is decreased, the resulting deformity, and the acuteness of the arthritis itself are controllable by means of plaster splints, used for intervals, and recurring rest for the joints in the best functional positions. It has been found that early use of rest, protection with supervised voluntary exercise periods, gives the quickest and best results as far as the joints are concerned. It has been quite encouraging to see the surprising improvement of the joints by anticipating the usual deformities.

In reviewing the atrophic cases, it seems evident that by anticipating the arthritis in the slender, unstable, visceroptotic, the arthritis itself can be best

controlled. Having followed many young women with such symptoms, and seen the marked improvement under systematic, continued building up with rest, corrective exercises and high vitamin diet, I believe that all these potential arthritides should be recognized and built up to robust health as far as possible early, before they have arthritis, when they come for general examination. The potential weakness common to the patient with atrophic arthritis can be corrected gradually and the arthritis in many cases prevented.

The method of treatment can be based on the following facts. That out of 37 cases of atrophic arthritis, 36 were in the D posture class,¹⁴ using the Harvard standard. They are all congenital ptotics, because of their anatomic type.⁹ Poor posture and visceroptosis together result in low vitality and diminished health.¹⁵ Our first attack is to correct the posture and thus give the viscera a chance to do more of their work. Second after thus securing better nutritional conditions, feed the patient the freshest of fruits, vegetables, milk, eggs, whole grains, and all kinds of mineral, vitamin rich foods, which are alkaline forming and not acid, as these people³ are extraordinarily acid in their sweat and saliva, and remain so for long periods. Third, complete rest with exercise in bed until they can continue the exercise up and about. Rest is essential. Fourth, sunlight, fresh air and outdoor exercise if possible. Fifth, regulation of the home activities to secure rest, leisure, relief from nervous strain and worry. Fatigue is probably the most dangerous factor in relapses as this means neglect of the essentials for health—posture, rest and sleep. Lowered vitality is followed by an increase in the previously noted symptoms.

No matter what the precipitating cause of the joint involvement is, no matter what the final cause of the atrophic arthritis is found to be bacteria (Cecil,¹⁶ Burbank,¹⁷ Crowe,¹⁸ etc.), or circulatory (Pemberton,¹⁹ Douthwaite²⁰), or intestinal (Fletcher²¹⁻²²), this poor physical condition and lack of resistance and instability allow these final factors to become active in the production of the joint changes. Many cases of atrophic arthritis show bone atrophy and joint destruction without subjective joint symptoms. Unless, therefore, something is permanently done to restore this inherent lack of vitality, to restore the circulatory stability, and to put the patient on a higher plane of health the removal of a focus, the use of a vaccine of rest, and dieting can be of temporary benefit only. The underlying condition remains as it always has been from childhood up. The goal to be worked for is permanent restoration if possible, of metabolic stability, so that the environment may be met with some degree of assurance. Oftentimes the atrophic equilibrium is so poor he cannot even cope successfully with any foreign proteid, vaccine, or endocrine stimulation.⁶

If we are to be permanently successful in the treatment of arthritis of this type, we would do well to look at it from a broad point of view, the same as we do with tuberculosis.

In tuberculosis, an exposed child is taken in hand at once and treated as a potential tuberculous patient until he is well and resistant. Time and care are expended in treating tuberculosis. Atrophic arthritis must be attacked in the same way. Recognize the potential atrophic with the hereditary possibilities, the type of anatomy, the vasomotor instability with the cold clammy

hands, the chronic fatigue, the lack of vitality, and treat him before any serious damage has been done. In this way it has been possible to prevent at least a few more cripples of the atrophic type. Even if we find a final precipitating cause of atrophic arthritis, we have still to deal with the fundamental physical condition which is back of the ill health of the atrophic patient.

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THE SYNOVIAL FLUID IN HEALTH AND DISEASE WITH SPECIAL REFERENCE TO ARTHRITIS

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THE purpose of this paper is to collect, classify and evaluate, the available data on synovial fluid in order to ascertain, first, whether the known properties of joint exudates can be utilized for clinical purposes, and second, whether further studies on fluid of joints are likely to be of value, and if so, in what direction such investigations should proceed. One is now able by a study of the physical, chemical, serologic and biologic characteristics of spinal fluid to acquire valuable data for diagnosis, prognosis and treatment of diseases of the central nervous system. The synovial fluid, on the other hand, has been neglected both by the internist and by the surgeon. Many studies on certain special phases of this problem have been undertaken but no concerted effort has been recorded to carry out complete investigations of the synovial fluid. This is surprising when it is realized that pathologic joint exudate is one of the most easily available body fluids that it can often be obtained in large amounts and that its withdrawal requires no expert technique, causes little discomfort and entails practically no risk to the patient provided that strict aseptic precautions are maintained.

From a detailed review of the literature on synovial fluid it was learned that much of the data concerning its physical, chemical and biologic properties has been recorded in numerical values. Because of this it seemed advisable, for the sake of brevity and of convenience in comparison, to tabulate the various findings. Tables I and II list the physical and chemical characteristics together with dates, names of investigators, numbers of cases and diagnoses. Tables III, IV, V and VI list the biologic data including Wassermann reactions, results of animal inoculation and of culture together with histologic findings. Table VII is a composite of the other tables.

For information concerning the formation of synovial fluid and of the synovial membrane and their relations to neighboring structures the reader is referred to the papers of Key¹ Mayeda² and of Franceschini.³ The cavity which is destined to contain synovial fluid is formed relatively late in embryonic life by a splitting of tissues which form clefts lined by mesenchymal connective tissue cells. These living cells have been referred to in the literature as endothelial elements, but the evidence is in favor of their being really connective tissue cells rather than endothelium. Forkner, Shands and Poston⁴ have suggested that they be called "mesothelial cells."

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TABLE I

PHYSICAL AND CHEMICAL PROPERTIES OF HUMAN SYNOVIAL FLUID A NORMAL, GENERAL EDEMA, VARIOUS DISEASES AT AUTOPSY, NONSPECIFIC EFFUSIONS, ACUTE SYNOVITIS, BURSITIS, SEPTIC ARTHRITIS, GONORRHOAL ARTHRITIS, SYPHILITIC SYNOVITIS, AND CHARCOT JOINTS

DIAGNOSIS	NO OF CASES	OBSERVER AND BIBLIOGRAPHIC NUMBER	DATE OF OBSERVATION	SPECIFIC GRAVITY	VISCOSITY 20° C	PRESSURE MM FL	WATER PER CENT	SOLIDS PER CENT	H ⁺	TOTAL PROTEIN	TOTAL NITROGEN	ALBUMIN	GLOBULIN	ALBUMIN GLOBULIN RATIO	SUGAR MILLIGRAMS PER 100 C C	SODIUM CHLORIDE	CHLORIDE	PER CENT	PER CENT	ICTERIC INDEX	GENERAL REMARKS
Normal	2	Mayer	1919	1.040						1.6											
Normal	8	Fisher	1923					4.41											1.95		Autopsy material
Normal	9	Seeliger	1926						8.2 8.4												Autopsy material
Various Diseases (No Arthritis)	30	Schneider	1925		3.9 1490																
General Edema	1	Boots and Gullen	1922						7.34												
General Edema	1	Cajori and Pemberton	1928							1.39		1.03	0.25	1.0					0.42		
General Edema	25	Wernke	1924	1.018	4.71		94.49 98.80	1.20 5.51	weak alk			0.45 3.90									
Serous Effusion	2	Ranke	1875			68															
Bloody Effusion	9	Ranke	1875			204 2720															
Hydrops Non specific	5	Habler	1928						7.21 7.58												
Effusion	16	Fremont Smith and Dailey	1926				93.7 96.1			3.63 6.33					72 119		351 408	19 33			

TABLE II

PHYSICAL AND CHEMICAL PROPERTIES OF HUMAN SYNOVIAL FLUID B TRAUMATIC EFFUSION, CHRONIC ARTHRITIS, ACUTE RHEUMATIC FEVER, TUBERCULOUS ARTHRITIS, ARTHRITIS OF SERUM DISEASE AND INTERMITTENT HYDRAETHROSIS

DIAGNOSIS	NO OF CASES	OBSERVER AND BIBLIOGRAPHIC NUMBER	DATE OF OBSERVATION	SPECIFIC GRAVITY	VISCOSITY 30° C	PRESSURE MM FLUID	WATER PER CENT	SOLIDS PER CENT	H ⁺	PER CENT			SUGAR MILLIGRAMS PER 100 CC	SODIUM CHLORIDE MILLIGRAMS PER 100 CC	N P N	MUCIN PER CENT	ICTERIC INDEX
										TOTAL PROTEIN	TOTAL NITROGEN	ALBUMIN	GLOBULIN				
Traumatic effusion	11	Habler	15 1928						7 13 7 50								
Traumatic effusion	5	Lasch	30 1928						7 39 7 68								
Traumatic effusion	22	Rostock	19 1929			0 340											
Traumatic effusion (bloody)	34	Rostock	19 1929			0 700											7 40 28 0
Traumatic effusion	13	Kling	18 1930														
Traumatic arthritis	1	Cajori et al	6 1926		3 3			6 25	7 58	4 18	0 71				40		
Chronic arthritis or traumatic effusion	8	Allison et al	7 1926							3 64 6 33					19 29		
Chronic arthritis or traumatic effusion	10	Allison et al	7 1926										58 103	584 674			
Chronic arthritis or traumatic effusion (during anesthesia)	6	Allison et al	7 1926											592 636	21 34		
Chronic arthritis or traumatic effusion	7	Allison et al	7 1926										89 116				

There is considerable discussion and uncertainty concerning the origin of synovial fluid. The following theories, among others, have been advanced: (1) It is the product of physiologic destruction and disintegration of tissues of the joint brought about by movement. (2) It is a transudate from blood or lymph vessels. (3) It is a mixture of transudate and disintegration products. (4) It is a secretion of the synovial lining cells. (5) Certain special cells are present in the so-called synovial membrane for the secretion of mucin or related substances. None of these theories has gained general acceptance.

An opinion is current, which appears to be entirely without scientific support, that synovial fluid is a "dead" fluid and that it really possesses no important functions other than that of lubrication. Some of the earliest chemical studies, on the other hand, as Tables I to VII show, demonstrated that this fluid not only contains a lubricating factor, that is to say, mucin or related substances, but also is rich in proteins, inorganic salts, and sugar. Strangeways⁵ in 1920 advanced additional evidence to show that synovial fluid possesses nutritive functions. He examined loose bodies in the joint which were composed of living growing cartilage and bone cells. These tissues had no other source for their nourishment than from synovial fluid. He believed that the primary cause of changes in certain types of arthritis was to be found not in the cartilage or bone, but in changes of the nutritive value of joint fluid. Cajori, Clouter and Pemberton⁶ studied synovial fluid extensively from the chemical point of view and concluded, "The adequacy of the synovial fluid for the nutrition of cartilage from the standpoint of carbohydrate and energy-yielding content is clearly indicated."

Alhson, Fremont-Smith, Dailey and Kennard⁷ found that in noninfected synovial fluids the sugar content was nearly always lower than in the blood plasma, whereas the protein was always lower and the chloride always higher than in the blood plasma.

NORMAL SYNOVIAL FLUID

Physical and Chemical Properties (Table I).—The normal synovial fluid of man is a transparent, highly viscous, almost acellular fluid about which very little reliable information is recorded. Because of the small amount of fluid contained within the capsule of normal joints, and because of the lack of interest in the synovial fluid generally, research in this field has been limited. The specific gravity is reported as 1.040, total solids as 4.41 per cent, total protein 1.6 per cent, mucin content 1.95 per cent and P_H as 8.2 to 8.4. These analyses have not been confirmed and they are, therefore, purely provisional values. Judging from the extensive work on the P_H of pathologic synovial fluid it seems that the P_H values of normal synovial fluid as recorded by Seeliger⁹ (Table I) are too high. It is much more probable that the P_H approximates that of the circulating blood.

Biologic Properties (Table III).—There are conflicting results concerning the cytology of normal synovial fluid. The total white cell count as recorded by Labor and von Balogh¹¹ is from 10 to 20 cells per cubic millimeter. They

found no red blood corpuscles Hammar,²⁰ working much earlier and with crude methods, found red corpuscles in the fluid of normal joints at autopsy. A recent study by Key² indicates that there are from 80 to 375 white cells and about an equal number of red blood cells per cubic millimeter in the normal synovial fluid of rabbits and children. These results were obtained from fluid recovered at operation, presumably under an anesthetic, and it seems entirely probable that the unavoidable trauma or congestion of vessels under such conditions may lead to an increased cellular content. Pending a confirmation of the above findings it may be assumed tentatively that normal synovial fluid contains ± 50 white cells per cubic millimeter.

As regards the differential cell count we have only the unconfirmed results of Key² which are recorded in Table III. He divides the cells into six general types, synovial lining cells, primitive cells, polymorphonuclear leucocytes, indeterminate phagocytic cells, clasmatoocytes and monocytes. He did not find lymphocytes. On the other hand, numerous investigators studying the cells of various pathologic fluids have often noted that lymphocytes are present. No other investigator has recorded the finding of so called "primitive" or undifferentiated cells in synovial fluid. In the discussion of the types of cells encountered Key makes the following statement: "Since it has been shown by Cunningham, Sabin and Doan that the primitive cells develop into monocytes, all clasmatoocytes, monocytes, indeterminate mononuclear phagocytes and primitive cells may be grouped together as the macrophage series." This is indeed confusing and would appear to be an unjustified assumption. Cunningham, Sabin and Doan³ believe that not only monocytes, but all of the white blood cells are derived from 'primitive' undifferentiated cells by a process of orderly differentiation. Moreover the above authors do not group together primitive cells, monocytes and clasmatoocytes as cells of the macrophage series. The terms 'macrophage' and 'clasmatoocyte' are quite synonymous and imply that these cells are phagocytic for large and small particles or for cellular debris. The primitive cell of Cunningham, Sabin and Doan³ on the contrary is undifferentiated and incapable of phagocytosis.

It would seem unnecessary that a new classification be adopted for the cells of synovial fluid. The classification of Key omits lymphocytes and includes primitive cells and indeterminate phagocytes. In these respects it differs from that of all other investigators.

Forkner, Shands and Poston⁴ described the living cells of the synovial fluid as they appear when stained by means of the supravitral technique with neutral red and Janus green. They found in the joint fluid representatives of all the white cells commonly present in normal blood and in addition macrophages and synovial lining cells or mesothelial cells. It is not essential for cytologic studies that the supravitral technique be used, but it has been well established by numerous investigators that this method provides the best available means for the differentiation of monocytes, lymphocytes and macrophages. Forkner⁵ has recently described in detail the exact method.

TABLE III

BIOLOGIC PROPERTIES OF HUMAN SYNOVIAL FLUID A NORMAL, SIMPLE EFFUSION, NONSPECIFIC HYDROPS, INTERMITTENT HYDRARTHROSIS, TRAUMATIC EFFUSION, TRAUMATIC ARTHRITIS, AND ARTHRITIS OF DYSENTERY

DIAGNOSIS	NO OF CASES	OBSERVER AND BIBLIOGRAPHIC NUMBER	DATE OF OBSERVATION	WASSER MANN		ANIMAL INOCULATION	CULTURE	CYTOLOGY							GENERAL STATEMENTS		
				BLOOD	FLUID			TOTAL COUNT	PERCENTAGE								
									R.B.C	W.B.C	GRANULO CYTES	LYMPHO CYTES	MONO CYTES	MACRO PHAGES		MESOTHELIAL CELLS	
Normal (autopsy)	1	Hammar	20 1894					+	+						+	Also noted presence of threads, fat drop lets, degenerating cells, membranes, elastic fibers, and tissue masses	
Normal (autopsy)	1	Labor and von Balogh	21 1919					0	10 20						+		
Normal (children)	1	Key	22 1928					80 375	80 375	0 12		42 84	4 28	0 7			Also noted primitive cells 0 10 per cent, and indeterminate phagocytes 3 29 per cent
General edema (without arthritis)	1	Labor and von Balogh	21 1919					0	0 20						50 50		
Acute general sepsis (without arthritis)	1	Labor and von Balogh	21 1919						±400						50		
Nonspecific hydrops	1	Labor and von Balogh	21 1919						±40,000								Cell count high during stage of increasing fluid
Nonspecific hydrops	4	Habler	15 1928				Neg										

Also noted presence of threads, fat droplets, degenerating cells, membranes, elastic fibers, and tissue masses

Also noted primitive cells 0.10 per cent, and indeterminate phagocytes 3.29 per cent

Cell count high during stage of increasing fluid

used in Doctor Sabin's laboratory for the application of the supravital technique to the study of blood and tissue cells

Pending further investigations it may be tentatively accepted that the white cell formula of normal synovial fluid is as follows Mesothelial cells ± 3 per cent, polymorphonuclear leucocytes or granulocytes ± 5 per cent, large phagocytic cells or macrophages ± 30 per cent, and monocytes ± 58 per cent Whether or not lymphocytes are present is still an open question

SYNOVIAL FLUID IN CONDITIONS OF GENERALIZED EDEMA WITHOUT ARTHRITIS

Physical and Chemical Characteristics (Table I) —The specific gravity is apparently between 1.008 and 1.018, considerably lower than normal The viscosity has been recorded in one case as 4.71 The water content ranges from 94.49 to 98.80 per cent, and total solids from 1.20 to 5.51 per cent The P_H has been determined in only one case and was 7.34 Total protein recorded in one case was 1.39 per cent The quantity of albumin has a wide variation from 0.45 to 3.90 per cent, and globulin in one case was only 0.025 per cent, giving an albumin globulin ratio of 41 Mucin in one case measured 0.42 per cent

Biologic Properties (Table III) —Bacteriologic studies in one case (not included in table) recorded by Boots and Cullen¹¹ were negative Labor and von Balogh²¹ make the statement, unsupported by protocols, that the total white cell count is either normal or below normal and that macrophages and mesothelial cells are present in about equal numbers They found no red blood corpuscles

NONSPECIFIC JOINT EFFUSIONS

Under this heading is included a heterogeneous group of cases which are recorded in the literature without definite diagnoses Most of the cases probably represent one or another type of arthritis Any conclusions from data of this sort must, therefore, be accepted with reservations

Physical and Chemical Properties (Table I) —The viscosity in 11 cases has varied from 2.91 to 4.79 Pressure measured in millimeters of water or of synovial fluid in 2 cases of serous effusion was 68 and 272 mm With bloody effusion the pressure was often much higher, from 204 to 2720 mm Water content from 93.7 to 96.1 per cent has been recorded in 16 cases The P_H was on the alkaline side in five cases, from 7.21 to 7.58 In one case in which there was a leucocyte count of over 13,000 cells per cubic millimeter and in which there was a low sugar content (8 to 10 mg per 100 c c) and in which rapid glycolysis was present, the P_H shifted to the acid side, 6.5 to 6.6 Total protein is increased in such cases, ranging from 3.54 to 6.33 per cent Albumin and globulin are present in concentrations of from 2.26 to 3.72 and from 0.31 to 2.45 per cent respectively The albumin globulin ratio varied from 1.2 to 8.8 Sugar in the recorded cases approximated that of the blood, from 72 to 119 mg per 100 c c except in the one case mentioned above in which the

sugar fell to 8 or 10 mg per 100 cc and the lactic acid content rose to 52 mg per 100 cc. The chloride concentration ranged from 354 to 408 mg per 100 cc as recorded in 16 cases by Fremont Smith and Dailey.¹⁶ Sodium chloride varied from 573 to 623 mg per 100 cc in nine cases reported by Cajori and Pemberton.¹ Nonprotein nitrogen measured from 19 to 33 mg per 100 cc in 25 cases. Mucin was decreased and varied from 0.41 to 0.65 per cent.

Some of the chemical constituents of synovial fluid have been determined only in isolated instances and these are not recorded in the tables. For example, Cajori and Pemberton¹ determined the fibrin content in three non-infected synovial fluids and found values ranging from 16 to 50 mg per 100 cc. In the fluid of one case of traumatic arthritis Cajori, Cronter and Pemberton⁶ found a CO₂ value of 55.2 per cent by volume. The urea nitrogen concentration was determined by Cajori and Pemberton¹ in four non-infected synovial fluids, and it varied from 13 to 18 mg per 100 cc. In six similar cases the amino acid nitrogen varied between 4.7 and 6.8 mg per 100 cc.

Biologic Characteristics (Table III).—Cultures of the synovial fluid were negative in 4 cases. It has been stated by Labor and von Balogh¹ that during the stage of increasing fluid in the joint the white cell count may rise to 40,000 per cubic millimeter.

INTERMITTENT HYDRARTHROSIS

Physical and Chemical Characteristics (Table II).—The only record available is a study of the hydrogen ion concentration in one case recorded by Boots and Cullen.²¹ They found a P_H value of from 7.37 to 7.47.

Biologic Properties (Table III).—Culture of the fluid in the above case was negative. Baker²⁴ recorded one case associated with Malta fever in which *B. Melitensis* was repeatedly grown in pure culture from the joint fluid. In this case the total white cell count of the synovial fluid on one occasion was 6,500 cells per cubic millimeter. Dopter and Tanton²⁵ record two cases in one of which all the cells present were lymphocytes and in the other, half of the cells were granulocytes and half lymphocytes. Shands has recently cultured three fluids, in one *Streptococcus viridans* and in the other two, staphylococci were isolated.

TRAUMATIC EFFUSION AND TRAUMATIC ARTHRITIS

Physical and Chemical Characteristics (Table II).—In one case the viscosity is recorded as 3.3. Rostock¹⁹ found the intra-articular pressure in 56 patients to vary from 0 to 700 mm of fluid. Total solids in one case of traumatic arthritis measured 6.25 per cent. P_H varies from 7.13 to 7.68 and total protein from 3.64 to 6.33 per cent. Total nitrogen in one case measured 0.71 per cent. Allison, Fremont Smith, Dailey and Kennard⁷ reported a series of cases including both traumatic effusion and chronic arthritis in which they found the sugar to be between 58 and 103 mg per 100 cc and sodium chloride between 584 and 674 mg per 100 cc. They found also that the chemical constituents of these cases were not materially changed when the patient was

under anesthesia In six anesthetized patients the nonprotein nitrogen of the synovial fluid varied from 21 to 34 mg per 100 c c The icteric index in 18 cases recorded by Kling¹⁸ varied from 7.4 to 28.0

Biologic Characteristics (Table III) —The Wassermann reaction in cases of traumatic effusion is uniformly negative unless the patient also has a positive Wassermann in the blood In such cases, although there is no evidence that the arthritis is syphilitic in origin, the positive reaction may be carried over into the synovial fluid of the blood Four such cases are recorded by Kling³² Habler¹⁵ cultured fluids from 11 cases of traumatic effusion with negative results There is no record in the literature of either total white cell counts or total red corpuscle counts in the synovial fluid of any case of traumatic effusion or traumatic arthritis There is one constant finding, however, that red blood corpuscles have been uniformly present, sometimes in large numbers, in some cases constituting 99 or more per cent of the total number of cells present Apparently the percentage of granulocytes or polymorphonuclear cells may vary from 1 or 2 up to 100 per cent of the white cells Lymphocytes have been recorded from 2 to 33 per cent Monocytes in one case constituted 5 per cent Mesothelial cells have varied from none up to 98 per cent of the colorless cells Shands²⁵ reported four cases in which polymorphonuclear cells predominated

CHRONIC NONSPECIFIC ARTHRITIS

Under this category are included the diseases which have been called chronic infectious arthritis, atrophic arthritis, hypertrophic arthritis, arthritis deformans, rheumatoid arthritis, etc No attempt will be made here to discuss the classification of arthritis or to subclassify the diseases coming under the general heading of chronic nonspecific arthritis

Physical and Chemical Properties (Table II) —Ranke¹⁴ found the intra-articular pressure in cases of chronic arthritis to be from 68 to 395 mm of fluid The specific gravity in one case has been recorded as 1.017 Viscosity in eight cases measured 2.7 to 16.7 In one case the water content was 93.08 per cent Total solids have varied from 5.28 to 7.62 per cent, a value lower than 6.0 per cent having been obtained in but one case of nine The P_H has been studied by six investigators, four of whom found values ranging from 7.0 to 7.58 Lash³⁰ recorded higher values of 7.64 to 7.90, while Seeliger⁹ recorded an even more alkaline reaction of 8.2 to 8.4 Total protein varies between 3.92 and 7.25 per cent, whereas total nitrogen ranges from 0.74 to 1.16 per cent The sugar content in fourteen cases measured from 73 to 132 mg per 100 c c Salkowski³¹ in 1893 found in one case a very high sodium chloride value of 772 mg per 100 c c More recent work on eight cases showed lower values of from 409 to 633 mg per 100 c c The nonprotein nitrogen values vary from 22 to 43 mg per 100 c c The mucin content is given in one case as 0.27 and in another as 0.38 per cent

The following chemical data are not recorded in the tables because they represent isolated determinations Salkowski³¹ in 1893, in the fluid of a case

of chronic arthritis, found 569 mg of cholesterol, 17 mg of lecithin and 282 mg of fat per 100 c c. Cajori, Crouter and Pemherton⁶ found lactic acid in the fluids of two patients represented by 13 and 28 mg per 100 c c. In sixteen cases these observers recorded that the CO₂ content ranged from 43.1 to 68.1 per cent by volume and in three cases calcium varied from 8.3 to 10.7 mg per 100 c c. The amount of uric acid in the synovial fluids of seven patients recorded by these workers was from 3.3 to 4.7 mg per 100 c c.

Biologic Properties (Table IV) —The table shows the history of the bacteriologic findings in the synovial fluid in this group of diseases. Various kinds of organisms have been isolated. Apparently the green streptococcus, the hemolytic streptococcus, and the gonococcus are the chief offenders. Forkner⁴⁵ and Forkner, Shands and Poston⁴ have recently reported the finding of gonococci in pure culture on repeated occasions in the synovial fluid and axillary lymph nodes of a case of chronic infectious arthritis. The latter writers in 1928 also reported the isolation in four other cases of green streptococci from both the joints and regional lymph nodes. They pointed out that the presence of the same organism in both the lymph nodes and joints pointed toward these organisms as at least one of the contributing agents in the etiology of their cases. Shands²⁵ in another series of 42 cases has extended this work and has cultured organisms from 26 of 42 cases (60.9 per cent). The recent work of Cecil, Nichols and Stansby⁴⁴ has given similar results.

There has been very little work on the cytology of joint fluid in chronic nonspecific arthritis. Three cases of syphilis reported by Chesney, Kemp and Baetjer,⁴² having chronic arthritis, presumably of a nonsyphilitic type, had a high percentage of granulocytes in the synovial fluid and the total white cell counts in the fluids of two of them were 1,500 and 2,480 cells per cubic millimeter. Forkner, Shands and Poston⁴ studied the cytology of the joint fluid in eight cases, in which bacteria were cultured and in twenty-two cases in which the synovial fluid was sterile. They found wide variations in both the total and differential counts in each series of cases, but demonstrated that the average white cell count and the percentage of polymorphonuclear cells in the bacteriologically positive fluids was considerably greater than in the sterile joint fluids. These findings have been extended and confirmed by Shands.⁵

ACUTE RHEUMATIC FEVER

Physical and Chemical Properties (Table II) —All that we know on this subject is that the P_H in a series of eight cases was from 7.27 to 7.42.

Biologic Properties (Table V) —Cultures of joint fluid in eight patients with acute rheumatic fever recorded by Boots and Cullen¹¹ were consistently negative. Lahor and von Balogh¹ stated that the white cell count of the synovial fluid was about 7,000 cells per cubic millimeter of which about 3 per cent were mesothelial cells and the remainder were granulocytes and lymphocytes. In 1900 Widal¹⁸ stated that the majority of cells were polymorphonuclears and this was confirmed by Swift.⁴³

TABLE IV
BIOLOGIC PROPERTIES OF HUMAN SYNOVIAL FLUID B CHRONIC ARTHRITIS

DIAGNOSIS	NO OF CASES	OBSERVER AND BIBLIOGRAPHIC NUMBER	DATE OF OBSERVATION	WASSER MANN		INITIAL INOCULATION	CULTURE	TOTAL COUNT		CYTOLOGY					GENERAL STATEMENTS
				BLOOD	FLUID			R B C	W B C	GRANULO CYTES	LYMPHO CYTES	MONO CYTES	MACRO PHAGES	MESOTHE LIAL CELLS	
Chronic arthritis (rheumatoid)	7	Schuller	33	1893			+	Small bacillus							Found diplobacilli in scrapings from lymph nodes and in synovial fluid
Chronic arthritis (infectious)	7	Chauffard and Raymond	34	1896				Negative							
Chronic arthritis (rheumatoid)	18	Binnatyne et al	35	1896				Small bacillus							Chiefly polymorphous cells
Chronic arthritis (rheumatoid)	1	von Dungen and Schneider	36	1898						+					
Chronic arthritis	7	Widal and Ravault	37	1900						+					Polynuclears predom inate
Chronic arthritis	1	Doelter and Tanton	23	1901						+				+	
Chronic arthritis	1	Juillard	26	1902							87	7	2	4	RBC 48 per cent WBC 52 per cent
Chronic arthritis (infectious)	2	Abadie	38	1902								+	+	+	RBC 60 per cent
Chronic arthritis (rheumatoid)	1	Poynton and Paine	39	1902			+	Diplococcus							Small number of poly nuclears
Chronic arthritis	2	Davis	40	1912										+	
Chronic arthritis (deformans)	4	Davis	41	1913				Negative							

TABLE IV—CONT D

[illegible]

TABLE IV—Cont'd

DIAGNOSIS	NO OF CASES	OBSERVER AND BIBLIOGRAPHIC NUMBER	DATE OF OBSERVATION	WASSER MANN		ANIMAL INOCULATION	CULTURE	TOTAL COUNT		CYTOLOGY PERCENTAGE						GENERAL STATEMENTS
				BLOOD	FLUID			R.B.C	W.C	GRANULO CYTES	LYMPHO CYTES	MONO CYTES	MACRO PHAGES	MESOTHE LIAL CELLS		
Chronic arthritis	1	Cecil et al	44 1929				Streptococcus viridans									Cultures from curet tings of joints
Chronic arthritis	1	Cecil et al	44 1929				Streptococcus nonhemolyticus									Cultures from curet tings of joints
Chronic arthritis	1	Cecil et al	44 1929				Diphtheroid									Cultures from curet tings of joints
Chronic arthritis	1	Boots and Cullen	11 1922				Negative									
Chronic arthritis	14	Shands	25 1930				Streptococcus viridans									
Chronic arthritis	2	Shands	25 1930				Streptococcus nonhemolyticus									
Chronic arthritis	1	Shands	25 1930				Streptococcus hemolyticus									
Chronic arthritis	4	Shands	25 1930				Staphylococcus aureus									
Chronic arthritis	4	Shands	25 1930				Staphylococcus albus									
Chronic arthritis	1	Shands	25 1930				Hemolytic staphylococcus aureus									
Chronic arthritis	16	Shands	25 1930				Negative									The total and differ- ential cell counts in the positive and negative joint fluids were essen- tially the same as in the cases re- ported by Forkner, Shands and Poston (4)

ARTHRITIS OF DYSENTERY

Physical and Chemical Properties—There are no data on this subject

Biologic Properties (Table III)—It has been pointed out that in the joints during the acute stage of this disease the total white cell count varies from 2,000 to 5,000 cells per cubic millimeter and that the granulocytes are more numerous than lymphocytes in the synovial fluid. In the convalescent stage the total white cell count is between 750 and 1,500 cells per cubic millimeter and lymphocytes may exceed granulocytes in number.

ACUTE SEPTIC ARTHRITIS

Physical and Chemical Properties (Table I)—The intraarticular pressure in cases of acute septic arthritis varied from 150 to 748 mm. of fluid. The P_H values were considerably lower in septic joint fluid from 6.19 to 7.24. Total protein in three cases varied from 3.56 to 6.92 per cent. Sugar was definitely depressed ranging in four cases from 19 to 43 mg. per 100 c.c. Sodium chloride varied from 556 to 577 mg. per 100 c.c. in three cases and nonprotein nitrogen from 23 to 27 mg. per 100 c.c. Icteric index in one case was 5.2.

Biologic Properties (Table V)—Chesney, Kemp and Baetjer⁴⁵ reported one case in which the total count was 1500 cells per cubic millimeter all of which were granulocytes. Culture of the synovial fluid or direct smear usually demonstrates the etiologic agent.

ACUTE GONORRHEAL ARTHRITIS

Physical and Chemical Data (Table I)—The P_H of the synovial fluid in one case was recorded as 6.97 and the icteric index in one case was 4.7.

Biologic Data—Cultures often reveal gonococci. No further studies are recorded.

TUBERCULOUS ARTHRITIS

Physical and Chemical Data (Table II)—The sugar content in two cases was 45 and 61 mg. per 100 c.c. and the P_H in one case 7.06.

Biologic Data (Table V)—Although animal inoculation and culture in many of these cases should be positive no records were found in the literature of this procedure having been carried out. The total white cell count in seven cases varied from 2,300 to 7,300 cells per cubic millimeter. One case showed 2 per cent granulocytes, 96 per cent lymphocytes and 4 per cent monocytes. In five cases the granulocytes composed from 50 to 70 per cent of the cells and lymphocytes from 10 to 40 per cent.

SYPHILITIC ARTHRITIS

There is no data recorded concerning a chemical or physical analysis of synovial fluid in syphilitic arthritis.

Biologic Properties (Table VI)—There has been some difference of opinion concerning the possibilities of obtaining a positive Wassermann reaction in synovial fluid with a negative reaction on the blood. Four such cases are recorded by Reschke and one by Chesney, Kemp and Baetjer.⁴² Todd⁴⁴

TABLE 1—CONT'D

	1	Parker	45	1928				Conococcus	3240	87	9	8	Also found gonococci in axillary lymph nodes
Gonorrheal arthritis (chronic infectious)	1	Aebard and Loeper	46	1900					+	2	96	4	
Tuberculous arthritis	1	Widal and Ravault	37	1900					+		+		Marked lympho cytosis
Tuberculous arthritis (early)	1	Widal and Ravault	37	1900					+	Predom inate			
Tuberculous arthritis (late)	1	Dopter and Tanton	23	1901					+		Predom inate		
Tuberculous arthritis	1	Dopter and Tanton	23	1901					+	+	+		Few Lymphocytes and polynuclears equal in number
Tuberculous arthritis	1	Julivard	26	1902					Few	36	56	8	
Tuberculous arthritis	2	Lewy	47	1921				Few	2300 6000	10 40	10 40		
Tuberculous arthritis	2	Lewy	47	1921					1500 7300	+	Predom inate		
Acute rheumatic fever	1	Widal	48	1900						+			Polynuclears pre dominate
Acute rheumatic fever	1	Lebor and von Bielow	21	1919					7000 ±	+	+		Polynuclears pre dominate
Acute rheumatic fever	8	Boots and Cullen	11	1922				Negative					High percentage of polynuclears
Acute rheumatic fever	1	Swift	49	1926						+			

TABLE VI--CONT'D

[illegible]

has likewise observed this phenomenon. On the other hand it is generally agreed that a joint fluid yielding a negative Wassermann reaction is frequently found in association with a positive reaction on the blood. Also it is apparent that in the presence of a positive reaction on the blood, a positive reaction on the synovial fluid is of no diagnostic significance because frequently in such patients there is no pathologic condition in the joints. Whether or not a positive reaction in an apparently healthy joint fluid of a syphilitic means that sooner or later such a case if allowed to go untreated will develop syphilitic joint disease has apparently not been determined. If one is allowed to draw an analogy here with what is known about spinal fluid under analogous circumstances it may be possible that a positive Wassermann reaction on fluid from a clinically sound joint of a syphilitic individual is of bad prognostic significance so far as the ultimate involvement of the joint in the disease process is concerned.

Chesney, Kemp and Baetjer¹³ were successful in the transmission of syphilis to rabbits by intratesticular injection of synovial fluid from three cases of syphilitic arthritis occurring during early syphilis. They were unsuccessful in transmission of the disease from three other patients, two of whom had syphilitic arthritis occurring late in the disease and the third suffered from tabes dorsalis and Charcot joints. Cultures from the joint fluids of all of the above patients were negative as were also dark-field examinations of the synovial fluid.

Total white cell counts in three cases of syphilitic arthritis in early syphilis varied from 17,600 to 24,000 cells per cubic millimeter. From 50 to 88 per cent of the cells were granulocytes and from 12 to 49 per cent were mononuclear cells. In two cases of syphilitic arthritis developing late in the disease, the synovial fluid contained 27 and 46 per cent of granulocytes respectively whereas mononuclear cells were 62 and 54 per cent. One case with a Charcot joint recorded by Chesney, Kemp and Baetjer¹³ showed a slight preponderance of polynuclear elements over the mononuclear cells. Ten patients with Charcot joints reported by Shands¹⁴ contained from 3000 to 3500 white cells per cubic millimeter in the fluid and showed a mononuclear preponderance.

CLINICAL VALUE OF STUDIES ON SYNOVIAL FLUID

The question now arises as to the clinical value of studies on the synovial fluid. Table VII summarizes some of the more important points on this subject. It is apparent from what has already been said that our information for the most part is very meager and largely unconfirmed. Even with these reservations certain important scientific and clinical facts are evident. The following significant points may be deduced from the tables:

1. A sugar content under 60 mg per 100 cc is almost always associated with infection in the joint.

2. A sugar content under 45 mg per 100 cc is strong evidence in favor of the presence of pyogenic organisms.

3. A P_H value in the neighborhood of 7.0 is strong evidence in favor of the presence of bacteria.

4 A P_H value under 70 is almost certain to be associated with the presence of pyogenic organisms

5 An ieteric index of over 55 is practically invariably a sign that trauma is playing or has played a significant role in the etiology

6 A positive Wassermann reaction in the joint fluid associated with a negative reaction in the blood is strong evidence in favor of syphilitic arthritis

7 A positive Wassermann in joint fluid with a positive in the blood may or may not be associated with syphilitic arthritis

8 A positive Wassermann reaction in the blood associated with a negative reaction in the synovial fluid probably represents good protection against the ultimate development of syphilitic joint disease

9 A high leucocyte count of 11 000 or more cells per cubic millimeter associated with 60 per cent or more of granulocytes in the synovial fluid of a patient with chronic nonspecific arthritis is likely to be associated with the presence of a positive culture of attenuated organisms

10 A leucocyte count in the joint fluid of 5 000 or fewer cells per cubic millimeter together with less than 50 per cent of granulocytes in a patient with chronic nonspecific arthritis is likely to be associated with a negative culture of the fluid

11 The presence of large numbers of red blood corpuscles in the synovial fluid of a patient with arthritis is evidence against chronic nonspecific tuberculous syphilitic or acute septic arthritis and is in favor of trauma as the etiologic agent

12 Animal inoculation is of value in the diagnosis of syphilitic arthritis occurring early in the disease

THE NEED FOR INVESTIGATION ON SYNOVIAL FLUID

Most of the information contained in the tables is purely provisional and subject to confirmation or change. Also one is forced to admit that very little is known about either the chemical composition or cellular content of normal synovial fluid. There is much need for the application of the methods of microchemical analysis to this problem and for the further study of the physiology and cytology of normal fluid under conditions of rest and after activity of the joints and in individuals of different ages.

Almost nothing is known of the chemistry and very little of the cytology of synovial fluid in the interesting and obscure condition of intermittent hydrarthrosis. The same may be said with perhaps even more emphasis concerning the synovial fluid in acute rheumatic fever arthritis of dysentery acute gonorrheal arthritis and tuberculous arthritis. In view of what is known concerning the cytology of the lesions of tuberculosis it may be predicted that the cytology of the fluid in tuberculous joints when studied with the supravital technique may be diagnostic. One would expect to find large numbers of monocytes typical epithelioid cells and perhaps epithelioid giant

VII

STUDY OF SYNOVIAL FLUID

P. H.	WASSER MANN		TOTAL WHITE CELL COUNT	RED BLOOD CORPUSCLES	DIFFERENTIAL COUNT					CULTURE	ANIMAL INOCULATION
	BLOOD	FLUID			GRANULO CYTES	LYMPHO CYTES	MONO CYTES	MACRO-PHAGES	MESOTHE LIAL CELLS		
7 4	Neg	Neg	± 50	Probably absent or rare	± 5	1	± 58	± 30	± 3	Negative	Neg
± 7 34	Neg	Neg	0 50	None				+	+	Negative	
7 21 7 58	Neg	Neg	Variable							Negative	
7 37 7 47	Neg	Neg			±	+				Neg or positive Strep viridans Staph albus Staph aureus	
7 13 7 68	Neg	Neg		Many	1 100	2 33	± 5		0 98	Neg or positive Strep viridans Staph aureus	
7 0 7 9	Neg	Neg	Bacteriologically Positive Fluids							Neg or positive Strep viridans Strep hemolyticus Strep nonhemolyticus Staph aureus Staph albus Gonococcus Diphtheroid	
			± 11 000	Rare	± 61	± 19	± 15	± 3			
			Bacteriologically Negative Fluids							Usually positive for infecting organism	
			± 4,800	Rare	± 48	± 27	± 21	± 3	± 1		
7 27 7 42	Neg	Neg	± 7 000		Predominate	+			± 3	Negative	
	Neg	Neg	750 5 000		Predominate in acute stage	Predominate in convalescent stage					
6 19 7 24	Neg	Neg	Often very high		± 99						
± 6 97	Neg	Neg	Often very high		Predominate					Gonococci may often be cultured	
± 7 06	Neg	Neg	2 300 7 300		+	+	1				Often positive
	Pos or Neg	Pos	17 000 24 000		50 88	12 49				Negative Dark field examination also negative	Positive
	Pos or Neg	Pos			24 46	54 62				Negative Dark field examination also negative	Neg
	Pos or Neg	Pos or Neg			± 56	± 44				Negative Dark field examination also negative	Neg

cells, the so-called Langhan's giant cells Syphilis of the joint may also show such changes, but can easily be differentiated by the history, Wassermann reaction, and by animal inoculation

It is very significant as regards the bacteriology of joint exudates that attenuated organisms can, by persistent, careful, patient investigation, be cultured from a high percentage of cases with chronic, nonspecific arthritis The recent contribution of Shands²⁵ in which the same types of organisms have been grown from Charcot joints, from cases of traumatic arthritis and from cases of intermittent hydrarthrosis would tend to support a theory that in the etiology of arthritis there are several factors involved One factor is undoubtedly injury to the joint structures whether that be by direct trauma, by trophic, metabolic or deficiency disturbances, or as the result of congenital defects Another important factor, at least in a fair proportion of cases, is an actual invasion by organisms of one or another type There is an urgent demand for further work in the biologic as well as the chemical analysis of synovial fluid which may help to prevent and to treat satisfactorily the commonest sort of ailment that man is susceptible to, chronic disease of joints

SUMMARY AND CONCLUSIONS

1 Physical, chemical and biologic data on the synovial fluid in health and disease have been reviewed in detail and tabulated for comparison

2 A study of the synovial fluid in arthritis provides important differential diagnostic points It yields information which approaches in importance that obtainable from the study of cerebrospinal fluid

3 The need for further research on normal and pathologic joint fluid is stressed and the direction for such investigations is indicated

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CHRONIC ARTHRITIS

I A BASIC DIET FOR CHRONIC ARTHRITIS

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NEW YORK, N Y

IMPORTANCE of the Subject—There is perhaps no subject to which the medical profession of today is giving much attention as to arthritis. This will undoubtedly continue to be the case for years to come, for research has proved that a relatively large percentage of the population is suffering from the disease, and its cure or mitigation is therefore a matter of vital social and economic significance. This and other articles to follow containing practical considerations of the problem of chronic arthritis are based upon the assumption that arthritis is, in many cases, curable, and that under the supervision of physicians who have specialized in its study it presents the same possibilities for a favorable prognosis as are found in any other curable disease.

The Purpose of These Articles—It is the plan in this series of articles, to present the results of many years of practical clinical endeavor in the treatment of arthritis. Many aspects of the etiology and therapeutics of the disease will be considered systematically. The rôle of diet in arthritis, the relation of the colon and colonic therapy, the importance of foci of infection, menopausal arthritis and its endocrinologic aspects, vaccine, sera and drug therapy, the employment of physiotherapy and hydrotherapy, posture, will all be considered, not as separate entities, but as correlating forces in the treatment of arthritis. No one type of therapy can be held forth as a panacea for every type of arthritis, for the reason that its etiologic roots are too deeply anchored in the warp and woof of the human mechanism. The successful treatment of arthritis demands that this be kept in mind. A broader understanding of the physiology, biochemistry, and bacteriology of the human mechanism is essential to the physician who is dealing with the problem of arthritis. Single-minded faddists have no proper place in the modern attack on this disease.

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History of the Disease—Chronic arthritis is not a new disease. Its historical frontiers date back to the Mesozoic Age, centuries prior to the earliest recovered human fossils. Because it etches its ineradicable imprint on bone a tissue easily fossilized and which defies the ravages of time, arthritis has been traced to the early Age of Reptiles, 15 000,000 years ago. Since that time the paleontologic evidence of the disease has become increasingly abundant. It is not possible in an article of this kind to present a complete history of the disease, but to portray arthritis adequately as it exists today it is important to consider briefly the high lights of its history in order to realize how deep rooted it is in the biologic history of man.

Arthritis was a well known and even common ailment in ancient Egypt. Flinders Petrie found undisputed evidences of the disease in the skeletons unearthed in the tombs of Gurob, which date back to 1300 B.C. While Hippocrates was elucidating his Aphorisms on gout 300 years before the Christian era, Erasistratus was employing modern hydrologic methods of treatment for arthritis. Two centuries later Pliny and Seneca were calling the ancient Romans to account for their riotous living and attributed the prevalence of arthritis to excesses of diet. An Les Bains was almost as popular a cure for arthritis at the dawn of the first century A.D. as it is today. Celsus and Galen advocated bleeding, purgations, and local applications to the joints of their second century patients.

Classifications—No other disease group has had as many attempts at classification as has arthritis. No attempt will be made here to give in detail the classifications of Charcot, Virchow, Sauvage, Cullen, Landre-Beauvais, and a host of others. In 1827 Seudamore first recognized the importance of the inflammatory changes in the fibrous tissue in articular and periarthritic structures, and two years later Cruveilhier for the first time clearly differentiated osteoarthritis as a distinct disease entity. Garrod in 1833 was the first to demonstrate an excess of uric acid in the blood of gouty subjects and in 1890 recognized two distinct types of arthritis. This classification still has considerable vogue. The ensuing years saw many futile attempts to bring order out of chaotic classifications and even today there is much confusion in nomenclature. This fact, added to the still obscure etiology of the disease, makes the subject an exceedingly difficult one to investigate and treat.

Goldthwait classified chronic arthritis into (1) infectious, (2) atrophic, and (3) hypertrophic.

In 1909 Nichols and Richardson, in a masterly monograph based on a series of 65 cases of chronic nontuberculous deforming arthritis, most of which was accompanied by pathologic examination of postoperative or autopsy specimens, simplified the classification of arthritis, on the basis of morphologic changes into two main types (1) proliferative or atrophic or ankylosing and (2) degenerative or hypertrophic or nonankylosing, corresponding respectively to the atrophic and hypertrophic classification of Goldthwait.

In 1922, the British Ministry of Health adopted a classification which in essence agrees with that of Nichols and Richardson, using however, the term rheumatoid arthritis for the atrophic type and osteoarthritis for the hypertrophic type.

There are at present at least seven different types of classification sponsored by recognized authorities. This flat difference of opinion bears eloquent witness to the obscurity which still seems to surround our knowledge of the etiology, pathogenesis and treatment of this disease.

Statistics—A study of the following data cannot fail to impress one with the tremendous urgency of the problem of arthritis.

Sweden 91 per cent of all cases causing "permanent pensionable invalidity" are due to articular rheumatism.

England Under an all-embracing classification of rheumatic disease, rheumatism is responsible for one-sixth of the entire industrial invalidity, necessitating an expenditure of approximately \$10,000,000 a year for payments of sick benefit and a loss of working time amounting to 3,000,000 weeks a year. In 1922 about 90,000 patients applied for medical advice for chronic arthritis. Of these about 40,000 were severe cases and 25,000 of moderate severity and 25,000 were early cases.

Denmark In 1923, 14 per cent of 7,297 persons receiving total invalidity pensions suffered from some form of chronic arthritis.

Germany Here Arnold Zimmer has demonstrated, from statistics of the State Insurance Institutions, an enormous invalidity attributable to chronic rheumatic disease. A Research Institute in Rheumatism, to work with the University of Dusseldorf, has been established.

This widespread prevalence of arthritis is just as evident in other European countries as in those quoted.

There are no complete accurate statistics available for the United States, but there is no doubt in the minds of many physicians who have hospital and clinic facilities that a great percentage of patients applying for treatment are sufferers from some form of arthritis. The consensus of opinion in the larger clinics in New York City is that about 20 per cent of patients applying for treatment are patients who have arthritis.

An Organized Crusade—In Europe, national and international organizations are actively engaged in the study of arthritis. The International Committee on Rheumatism, with headquarters in Amsterdam, is directing the problems of the study, treatment, and prevention of arthritis. In this country, in 1925 the Hospital for Ruptured and Crippled of New York City organized an Arthritis Clinic, the first to be devoted solely to the study and treatment of arthritis. A group of specialists in the various branches of medicine and surgery were assembled in a single unit, aided by the laboratory and technical staff of the hospital, to determine the relationship of their specialties to the problem of arthritis. In 1926 an American Committee under the chairmanship of Dr. Ralph Pemberton was formed to cooperate with the European groups in the crusade against arthritis.

The result of this awakened interest has manifested itself in the altering of the prognostic viewpoint. For many years this disease was regarded as hopelessly chronic, both by the profession and the laity, and this pessimistic attitude led to an apathetic indifference on the part of the profession to the consideration of the etiology of the disease and its many legitimate methods of therapy. For years patients were thus driven to cultists and faddists who

have profited by the lack of interest on the part of the medical profession with respect to arthritis. The disease is admittedly a difficult one with which to deal. Its classification is still under consideration, its etiology obscure, its stubbornness in yielding to treatment notorious. These factors have long constituted an efficient barrier to the interest of the practitioner, who often chooses to devote his time and energy to the more spectacular and perhaps better understood fields of his art in which results are more easily achieved.

This unfortunate situation is, however, rapidly being corrected. Through the influence of the organized groups both here and abroad clinics for the exhaustive study and treatment of arthritis have been established in nearly all the large cities of this country. These clinics, arthritis units, combine the skill of the physician, general surgeon, orthopedic surgeon, roentgenologist, otolaryngologist, gynecologist, urologist, dentist, physiotherapist together with trained laboratory technicians and nurses to provide for the patient a formidable armamentarium in the battle against arthritis. Able research workers are giving to the profession the results of their intensive efforts in monographs and in more pretentious volumes.

Thus the entire attitude of the profession and the laity is undergoing a change from that of regarding arthritis as involving a hopeless painful invalidism, to a more optimistic view. Perhaps there is no one element more essential than this change of attitude for successful treatment of this dreadful malady.

Causes of Arthritis—It is generally admitted that arthritis may find its cause in dental or tonsillar sepsis or in infections of the paranasal sinuses, gall bladder, appendix, prostate, cervix or adnexa. These foci may, per se, be the causative agent, or, as considered by many eminent students of the disease, merely constitute the spark which sets the fire raging. Heredity, disorders of metabolism, pregnancy, the menopause and intestinal disorders, vitamin or glandular deficiency are all etiologically important. The American Committee for the control of rheumatism, as quoted by Pemberton, its Chairman, has placed itself on record as 'believing that the underlying causes of arthritis are often further to seek, and are to be found in part at least in that background determined by heredity, constitutional make up, the equilibrium of the nervous system, chemical, and other toxins of imponderable nature, and finally the conditions of the environment. Any of these causes may exist singly or in combination and their presence must be established unequivocally and treated on their own merits. This demands a thorough and careful work up of each case, often necessitating the consultation of specialists to establish the presence or absence of many of the contributing factors in the disease.'

Classification Used in These Papers—While the classification of arthritis on a morphologic basis as delineated by Nichols and Richardson, has accomplished much to clarify the issue, yet for practical clinical consideration it was found wanting. From the standpoint of etiology and as a guide to the treatment, it was decided by the authors to adopt the following classification: (1) infectious, (2) metabolic, and (3) mixed.

Under the first group were placed all cases in which demonstrable foci of infection were the undoubted etiologic factors. The second, or metabolic group

comprises cases in which heredity, disorders of metabolism, intestinal disorders, vitamin or endocrine deficiency, pregnancy or the menopause seem to be the dominating etiologic factor and in which no foci could be demonstrated. In this group gout is considered as a form of metabolic arthritis. In the third group, in which the majority of cases fall, are placed cases which show characteristics of both the foregoing classes. Quoting Glover in his *Report on Chronic Arthritis* for the British Ministry of Health: "For example, suppose the diagnosis of gout, the most undoubtedly metabolic disease of this group, the second duty, the search for the infecting focus, is a claimant, as ever, for in a large proportion of cases of gout an infective focus plays an important rôle in activating the metabolic process and in precipitating attacks." Of course, these groups are further classified as to whether they are acute or chronic. This purely clinical classification will be retained throughout this series of articles.

SOME PRACTICAL CONSIDERATIONS IN THE FORMATION OF A BASIC DIETARY FOR ARTHRITIC PATIENTS

Glover, in a *Report on Chronic Arthritis* for the British Ministry of Health in 1928, makes the following statement: "Diet is a most important part in the treatment of chronic arthritis. The indications are more various than in almost any other form of treatment."

No absolute rule can be laid down regarding the diet. The special features of each case should receive careful study. Simplicity and moderation are of the utmost importance.

After an exhaustive and careful perusal of the literature on the regulation of diet in arthritis and after many years of dietary regulation of hundreds of clinic and office patients, it was found that the ideal basic diet for arthritic patients should have the following characteristics:

- 1 Low caloric value
- 2 Low carbohydrate value
- 3 Low protein value
- 4 Low purin value
- 5 Adequate vitamin content
- 6 Elasticity
- 7 Availability

1 Low Caloric Value—In the Third Century A.D. Caelius Aurelianus, who affirmed that arthritis was caused by heredity, indigestion, over-drinking, debauchery, and exposure, advocated an abstemious dietary for arthritis. Since then there has been a consistent agreement of authorities that a low caloric intake is advisable, even necessary, to the successful treatment of arthritis.

It has been abundantly proved by experience that in most patients with arthritis a sharply reduced diet, almost a starvation diet, is beneficial in bringing about greater freedom of movement, comfort of the patient, and actual reduction of either high uric acid or sugar or both in the blood. Dr. Ralph Pemberton undoubtedly deserves the major portion of credit for drawing the attention of the medical profession to this fact.

Clinical experience of the authors agrees with that of many eminent students of arthritis that in the vast majority of cases there is an optimal level of caloric intake, definite for each individual below which the symptoms of the disease tend to decrease and above which they are aggravated and become persistent. By careful observation this level of caloric intake must be adjusted for each individual. The medical profession is apt to forget that no two individuals of the human race are any more alike in other respects than they are in their finger prints.

Kraus, Bruyseh and Mueller observed a general and considerable improvement in the cases of chronic arthritis in Germany during the war when the food shortage was acute.

Again, it has often been shown that patients with chronic arthritis, who come to operation for some intercurrent pathology, are definitely relieved of their arthritic symptoms during their postoperative convalescence with its concomitant restriction in food intake. This improvement is often mistakenly credited to the operation rather than to enforced starvation.

Drastic reduction in caloric intake in average uncomplicated cases of arthritis is perfectly safe provided and only provided the course of the patient's progress and weight is carefully supervised. Additions to the dietary can and should be made as rapidly as the condition of the individual patient warrants. In this manner it is possible to bring the diet up to the optimum level at which the patient's metabolism operates, and at which he is best able to carry on with a minimum of discomfort on the one hand and the maximum permissible energy on the other. In asthenic individuals or in patients whose arthritis is complicated by nephritis, hypertension, diabetes, etc., the dietary must be controlled with these concomitant factors in mind.

Regardless of the etiologic factors operating in any individual case it is certain that the metabolism of the sufferer from arthritis has been either overloaded or impaired. This is obvious by the very nature and symptoms of the malady. This metabolic deficiency may be due to a disturbance or interference in the capillary circulation as recently pointed out by Pemberton and his co-workers, or it may take on a much larger physiologic and biochemical aspect with probable involvement of the sympathetic nervous system. In any event by lowering the caloric intake and thereby relieving the metabolic burden the tissues of the body are given an opportunity to relieve themselves of this overload and to operate on a more nearly normal metabolic level. It seems logical to infer that when the organism is laboring under an increased metabolic burden it is rational therapy to lighten that load.

Fats are not inimical to the arthritic patient but since even small amounts will raise the total caloric value their careful restriction within the caloric limits of the diet is desirable.

The Colon—The importance of the role of the colon in arthritis will be dealt with in greater detail in a succeeding article but it is necessary to call attention here to some outstanding facts. It is generally conceded that arthritis may often be caused by bacterial proliferation in the body particularly in the colon. A reduction of caloric intake must of necessity tend to reduce the volume of the colonic content which acts as a most excellent culture medium for

bacterial growth. Thereby is reduced not only the number of bacteria in the colon but also those products of putrefaction generated by the action of bacteria on the feces, which may cause added mischief to an organism already physiologically below par. This is especially true where the reduction of caloric intake is considerable.

In the last decade a great deal of attention has been centered upon the intestinal tract, especially the colon and its relation to arthritis. Colonic therapy is becoming an increasingly employed method in the management of arthritis, and rightly so, for there is no doubt that, in a great number of cases, this mode of treatment is highly successful. It is therefore evident that it would be irrational to concentrate on the removal of undesirable and noxious intestinal contents without first regulating the intestinal intake in the matter of food. The character and amount of colonic content is determined primarily by the nature of the food ingested. Fletcher and Graham have recently demonstrated concomitant colonic abnormalities in patients with arthritis who were markedly improved by dietetic treatment with coincident improvement in the arthritis.

It is appropriate at this point, before beginning an analysis of blood chemistries of patients with chronic arthritis, to emphasize the fact that by far the greater majority of patients encountered in clinic and private practice have been under some form of medical lay or self-imposed régime, for some length of time prior to admission. This has included such forms of therapy as baking and massage, Hot Spring cures, dietary regulation (especially meat restriction) and the taking of drugs of the emelophen or salicylate groups which, as is well known, stimulate the kidneys to eliminate uric acid. It would, therefore, be difficult to estimate the blood chemistry values of these patients prior to such therapy at the onset of symptoms.

2 Low Carbohydrate Value—In a careful survey of about 1200 case histories and charts of patients with arthritis, both in clinics and office practice covering about five years, it was found that a great number of these patients had high blood sugar on admission. In the past five years of 425 consecutive patients suffering from arthritis seen in office practice who had blood chemistries done, 136 or more than 32 per cent had blood sugar values of 120 mg or more. Of 732 clinic patients with arthritis, 278 or 32 per cent plus, had blood sugar values ranging above 120 mg, which was considered the upper limit of normal.

BLOOD SUGAR

OFFICE CASES	110	120	130	140	150	160	170	180	190	200	Total cases
Below 110 mg	119	129	139	149	159	169	179	189	199	and up	
152	133	80	27	9	12	4		3		1	421

HOSPITAL CASES*

Below 110 mg	110	120	130	140	150	160	170	180	190	200	Total cases
	119	129	139	149	159	169	179	189	199	and up	
285	169	165	63	19	10	7	1	4	2	7	732

*These cases were seen in the Clinic for Arthritis, Hospital for Ruptured and Crippled, New York.

None of the patients proved to be diabetic. It was further noted that as treatment progressed and the symptoms cleared up, the blood sugar values

tended to approach normal. This was true only when patients were rigorous in the observance of their diet. Deviation from dietary regulation was attended with a recurrence of symptoms and a return of the blood sugar values to their former high levels. Blood samples were all taken in the morning and determinations were made immediately after the blood was obtained.

In Pemberton's article on Arthritis in *Nelson's Encyclopedia*, the following appears: "It appears at present useful, in undertaking treatment on the basis of reduced caloric intake to reduce the carbohydrates most, the proteins somewhat less, and the fats least of all, although it is also essential that the total caloric intake be reduced to a certain definite level. Carbohydrates constitute the chief caloric yielding substances of our diet under average conditions of life. Any attempt to reduce the total calories must therefore be with carbohydrates."

There is a general agreement among students of arthritis, that with many patients suffering from this disease there is a delay in the removal of the sugar ingested from the circulating blood. This lowered sugar tolerance is more or less variable with the degree of activity of the arthritic process. That it is not due to pancreatic deficiency was demonstrated by Cecil, Barr and Du Bois, who showed by calorimetric studies that the carbohydrate metabolism of patients with arthritis proceeds normally. Arthritis is not a part of the diabetic syndrome even though infections frequently occur in it. Rice demonstrated that the sugar tolerance curves of chronic arthritis and mild diabetes were similar and that the presence of some common factor was thereby suggested, but that they are distinct entities is shown by the fact that in arthritis there is no tendency to the formation of acetone or diacetic acid in the urine. The exact nature of the physiologic dysfunction responsible for this delayed sugar removal is not well understood. Pemberton believes that this delay is "brought about by some influence which causes vasoconstriction or some comparable state of the finer blood vessels in some of those tissues whose function it is to remove glucose, oxygen and presumably other substances from the circulatory blood."

Arthritis is not the only nondiabetic disease in which a delayed sugar removal is encountered. It is also found in certain (1) thyroid dysfunctions, (2) furunculosis and eczema (3) neuroses and (4) unrecognized inflammatory processes or focal infections but this phenomenon is more frequently coincidental with arthritis than with any other disease. This is especially true in cases in which demonstrable foci of infection occur, and furthermore it was shown by Pemberton that the rate of sugar removal showed an abrupt change toward the normal level after removal of the apparently causative foci of infection. There are, however, a certain number of patients with arthritis who show a lowered sugar tolerance in whom, even after the most painstaking investigation no foci can be demonstrated. It is also true that this lowered sugar tolerance is susceptible to rigid dietary regulation even in the presence of a surgical focus.

Symptomatic improvement of patients with arthritis who have demonstrated delayed glucose removal is accompanied by a return to approximately normal tolerance levels. It would seem probable in view of the foregoing observations, that a restriction of carbohydrate intake might tend to lift the burden

from the system which had demonstrated a physiologic dysfunction in the nature of disturbed rate of carbohydrate removal

It is common knowledge that patients with gout are frequently subject to an amylaceous dyspepsia. This type of carbohydrate indigestion has often been encountered by us in treating patients with metabolic arthritis, and it has been our experience as well as the common experience of other workers that this type of case is most frequently aided by a restriction of carbohydrate intake.

3 Low Protein Value—After carbohydrate reduction, the next important foodstuff to be considered is protein. Experience with dietary regulation of arthritis proves that a low protein intake is desirable. The reduction of protein should be accomplished by curtailing those foods which are subject to putrefactive decomposition in the colon. The chief offenders in this group are meats and fish. However, it is essential to preserve for the patient the minimum protein allowance of from 0.7 to 1.0 gram per kilo of body weight. This protein should be chiefly in the form of milk, eggs, cheese. Meats or fish may be included in the diet once a day or preferably once every other day, only to make the diet more acceptable to the patient and to secure his cooperation. In the zeal to eliminate the disease the individual is often forgotten. Ruthless dietary restriction will more often than not be provocative of antagonism on the part of the patient, who very often, especially in the metabolic cases, has developed a life-long habit of indulgence. If, by the inclusion in the dietary of carefully selected and restricted meats and fish, the monotony can be relieved, the hearty cooperation of the patient secured, more progress will be achieved than by causing resentment by strict unrelenting dietary control. The success of dietary regulation depends a great deal on persistence. Often, after months of treatment with drugs, irrigations, bakings, massage, vaccines, focus eliminations, etc., when apparently nothing has been accomplished in the way of clinical improvement, patience and perseverance in the dietary will bring definite amelioration. In order to be assured of the patient's cooperation in this long extended period of dietary control the diet must be, within limits, acceptable to the patient and practical in its home preparation or other availability.

4 Low Purin Value—Another important aspect of the protein regulation in the treatment of arthritis is its relation to the problem of uric acid and purin metabolism. In Glover's report on arthritis to the British Health Ministry the following statement is found: "It appears well established that the uric acid in the blood is increased in practically every case of gout and in many cases of osteoarthritis." J. Race has recently published the results of the examination of the blood in 662 patients in the Devonshire Hospital, Buxton, showing that there was marked increase in 100 per cent of cases of gout and in 79 per cent of male patients with osteoarthritis and 58 per cent of female patients. Maizels and Payne found "the blood uric acid abnormally high in 8 out of 15 cases of rheumatoid arthritis investigated at Guy's Hospital in 1927." Fohn and Davis in 1915 reported high uric acid findings in arthritis. Pratt in 1918 and Horowitz in 1926 found high blood uric acid values in nongouty arthritis. These results have been more than adequately substantiated in the experience of the authors, especially in cases falling in the metabolic or mixed groups. In a

series of 731 consecutive cases of arthritis seen in clinic practice at the Hospital for Ruptured and Crippled 381 or more than 50 per cent showed a decided increase in blood uric acid levels, in spite of the fact that more than 90 per cent of these patients had been on dietary restrictions with especial reference to meat and had been taking salicylates or some form of cinchophen both of which tend to lower blood uric acid values. The upper limit of normal for blood uric acid was set at 3.5 mg per 100 cc. Of these 731 patients 149 or about 20 per cent were males, and 582 were females, or 24 per cent and 75 per cent respectively.

In a series of 425 consecutive cases of arthritis seen in office practice, 250 or more than 59 per cent of the patients showed increase in blood uric acid. Of these 425 patients 138 or 32 per cent were male and 287 or 67 per cent were female. And what is strikingly important is that in the vast majority of clinic and office cases of arthritis, more especially in the metabolic types it has been found that the amount of clinical improvement varies with the blood uric acid value obtained as a result of treatment. Symptomatic improvement proceeds as the blood uric acid values approach normal levels. This phenomenon has been encountered so frequently as to make it almost possible to utilize the blood uric acid values as a guide to the patient's progress. It is to be remembered that the clinic and office patients mentioned above represent consecutive cases and all types, metabolic, infectious and mixed and were all ambulant cases. In this country one does not encounter the high blood uric acid figures found so frequently in gout, because pure gout as seen in England and the Continent, is comparatively rare in the United States.

URIC ACID

	BELOW 2.5 MG	2.5-2.9	3.0-3.4	3.5-3.9	4.0-4.4	4.5-4.9	5.0-5.4	5.5 AND UP	TOTAL
Office cases	12	42	121	112	96	25	9	8	425
Hospital cases	29	86	235	172	151	48	6	4	731

In view of these facts it would be intelligent therapy to limit the intake of protein not only because by its specific dynamic action it increases the metabolic load, but, according to McLester also because it increases the output of uric acid, and because purin bases which are the precursors of uric acid are derivatives of the metabolism of certain particular proteins.

It is an erroneous assumption that uric acid is a product of the metabolism of all proteins. It is only from the proteins which are rich in these compounds of nitrogen and phosphorus known as nucleins or nucleoproteins and which in their decomposition yield nucleic acid that uric acid and purin bases are derived. Nucleins, which as the name implies are derived from the nuclei of disintegrated cells, come from two sources. First from cell nuclei ingested in some kinds of food (exogenous nucleins) and secondly endogenous nucleins from the cells of the body which are constantly undergoing destruction as the result of general tissue activity. In the normal course of events nucleins both endogenous and exogenous are broken down by a series of ferments first into intermediate purin bodies such as guanine, adenine, hypoxanthine and xanthine and eventually into uric acid and urea in which two forms they are ultimately excreted. These proteins are found in greatest abundance in animal tissues.

made up largely of nuclei, i.e., liver, pancreas, thymus, kidneys. Other food stuffs which yield high uric acid values are, anchovies, sardines, herring, carp, lentils, and squab

PURIN CONTENT OF VARIOUS FOODS

(J. Schmid and G. Besson)

100 GRAMS	URIC ACID IN GRAMS
Beef	0.111
Veal	0.114
Mutton	0.078
Pork	0.123
Liver	0.279
Kidney	0.240
Sweetbreads	0.990
Chicken	0.087
Herring	0.207
Sardines	0.354
Anchovies	0.465
Spinach	0.072
Shell beans	0.081
Lentils	0.162
Oysters	0.087
Boiled ham	0.075
Fresh salmon	0.072
Lobster	0.066

It is this type of protein, that is responsible for high exogenous purin values, whose intake it is wise to curtail. It has been frequently demonstrated that the feeding of large amounts of nucleoproteins has been followed by an attack of gout. Dr. George Minot has reported that many of his cases of pernicious anemia treated successfully upon a large liver diet have developed hypertrophic arthritis. Quoting Llewellyn, "Even if we entirely eliminate all purin substances by restricting the diet to purin-free foodstuffs (bread, milk, cheese, eggs, and butter) purin in the form of uric acid is still excreted in the urine. To this variety the term endogenous purin is applied, for the continued excretion of purin on such a diet is explicable only on the view that they are derived from the wastes of the tissues, the daily wear and tear of the cells. In other words, it is the outcome of the katabolism of the nucleoproteins of the body tissue."

It would indeed be foolhardy to add to the food intake, purins which act only to increase the metabolic burden of an organism whose blood uric acid is in many cases abnormally high and which is already producing and excreting uric acid by the katabolism of its own tissues.

We are well aware of the fact that this study of the blood chemistry of chronic arthritis revealed no abnormally high blood sugar or blood uric values. The contention is not made that high blood uric acid or sugar cause arthritis, but they do indicate a disordered metabolism. This added to perhaps colonic stasis, or menopause, or infection or any of the aforementioned possible etiologic factors is sufficient to precipitate an attack. The contention is not made that proper diet alone will always or completely alleviate arthritic symptoms, but the successful treatment of arthritis will and must include dietary measures to restore, as far as possible, the normal metabolism as reflected in the blood sugar and blood uric acid. Failing this, all other measures directed to any other concurrent etiologic factor are liable to failure.

5 *Adequate Vitamin Content*—In 1927 M. J. Rowlands, in a communication to the Royal Society, developed the thesis that rheumatoid arthritis is a deficiency disease due in part to an inadequate Vitamin B content. Rowlands placed his patients on a concentrated Vitamin B diet and reported considerable clinical success from the treatment. H. A. Ellis also contends that rheumatoid arthritis is a deficiency disease, but considers the deficiency as a biochemical problem concerned with the utilization of phosphoric acid and its radicals, so that a deficiency of both Vitamin B and D is associated with it. Lovell Langstroth, in a recent examination of American dietaries based on a careful study of 501 general cases with controls, makes an eloquent plea for the inclusion of "protective foods" in the dietary because of their vitamin content. In this study it was demonstrated that the average American diet was "low in vitamin and residue, high in calories, in carbohydrate and in its ratio of acid to alkaline ash forming foods and contained a large proportion of concentrated foods." In the disease groups studied were hypertension, myocardial degeneration, arteriosclerosis, arthritis, chronic gastrointestinal disease, diabetes and the occasional unexplained headache. Incidentally 75 cases of arthritis were studied, of which 65 were degenerative arthritis, 8 proliferative arthritis, and 2 infectious arthritis. Many of the 65 cases presented only early changes manifested by tenderness and aching of the joints.

The conclusions arrived at on the basis of these investigations were interesting and important. It was found that 74 per cent of patients with degenerative or proliferative arthritis, the metabolic group, were improved or completely relieved by diet containing the required protective foods. The cases of infectious arthritis were improved by elimination of the food. The loss of weight occasioned by the dietary was considered as a possible factor in the relief afforded, especially in the lower weight bearing extremities, but the major role of the quality of food was demonstrated by the general improvement evident in the functions of the alimentary and circulatory systems as well as objective changes in the superficial tissues surrounding the affected joints.

The protective foods which are so rich in vitamin content are eggs, milk, fruit, vegetables and especially lettuce. Langstroth recommends a diet of 2,147 calories of which 70 per cent are these protective foods.

We have long been in full accord with these observations with respect to adequate vitamin content in the dietary of patients with arthritis, and in the basic diet advocated necessary provision has been made for ample representation of the protective vitamin containing foods. They have instituted a clinical study of the value of diets rich in Vitamin B, using well known commercial preparations which are known to contain relatively large amounts of this Vitamin B. These substances are used in conjunction with the diet, and the results so far, are gratifying—although it is too early to make any definite conclusions. Another series of clinical experiments using liver extracts is under way and will be reported on at a later date.

A great deal has been written about the role of vegetables in a diet for arthritis. It is axiomatic that the best diet for any disease entity is the one which causes the least indigestion. All embracing generalities such as "eat all green vegetables," "eat all vegetables which grow above the ground," etc.,

have been recommended. Cabbage, brussels sprouts, cauliflower and broccoli are all almost purin free and meet with nearly all requirements, but their effect on the digestion and elimination of many patients is so distressing as to make their ingestion at the beginning of treatment undesirable, even noxious. Spinach, beans, peas, lentils have relatively high purin values and should be partaken of sparingly. They may be utilized later in the course of dietary control as addenda. Potatoes, turnips, carrots, rice, parsnips and beets have relatively high carbohydrate values and should not be included in a basic diet. They also may be added later in treatment, although parsnips and turnips might well be ignored entirely because of their notorious association with indigestion in certain cases. It is therefore advisable to select carefully the vegetables to be placed on a dietary for arthritis rather than to allow a wide choice in the kind and amount of vegetables to be used.

The inclusion of fruits, especially fresh fruits, in a dietary for patients with arthritis is essential. The popular bogey of "fruits produce acid" has long since been robbed of its terror. They form, when confined within the caloric and carbohydrate limits of the diet, a most valuable adjunct to the diet because they contain no purins, are easily digestible, make the diet more attractive and palatable, lend bulk, without too greatly increasing the caloric value, and form a valuable source of vitamins.

6 *Elasticity*—It must be borne in mind that concomitant pathology is often found in patients with arthritis. Associated with arthritis, the disease entities often encountered are arteriosclerosis, hypertension, chronic nephritis, myocardial degeneration, cardiac valvular disease, obesity, diabetes and chronic gastrointestinal disease.

Any basic diet prepared for patients with arthritis must, of necessity, be so elastic as to be readily adaptable to any concurrent pathology which the patient may present.

7 *Availability*—The diet must be so contrived as to be easily available in restaurants, hotels and trains and must be easily adapted to preparation in the home without upsetting the normal kitchen regime. Any deviation from these requirements will make it difficult to secure whole-hearted cooperation from the patient.

The diet herewith presented is a basic diet which meets, in so far as possible and practical, the foregoing requirements, and is one on which practically any type of individual may carry on for a short period at least. Any patient with arthritis, regardless of weight or condition, may be placed on this diet with impunity for a period of ten days. During this interval the patient is most carefully observed and the diet then altered as the progress, general condition, weight, blood chemistry, and concomitant pathology of the patient indicates.

It might be well to note here that this diet is utilized in conjunction with other indicated procedures such as colonic irrigations, vaccine therapy or physiotherapy. It was not deemed wise to curtail this diet below its present caloric levels, considering the strain placed on the patient by these other therapeutic measures.

If the diet is to be used without other concurrent more or less strenuous form of treatment, it would perhaps be wise to limit the protein intake below the level indicated in the diet.

BASIC DIET

BREAKFAST

	PRO TEIN	FAT	CARBO HYDRATES	CAL- ORIES	URIC ACID	PROTEC TIVE FOODS	NONPRO TECTIVE
Choice of one of the following fruits							
One orange sliced or as orange juice no sugar	62	23	87.1	96		96	
or							
Grapefruit (half) no sugar or	97	56	124.1	139		139	
Strawberries	51	60	37.3	48.4		48.4	
Raspberries	34		42.3	46		46	
Blackberries	53	93	44.7	59		59	
(3 h tbsp no sugar)							
Two eggs any form except fried	54.2	111.6		166		166	
Bread—one slice thin toasted, white or whole wheat	11.3	3.6	65.3	80			80
Butter one pat 15 grams	0.6	118.6		119			119
Beverages one glass whole milk	29.8	81.8	45.1	157		157	
or							
One cup of coffee or tea (¼ cup of milk 1 tbsp cream, 2 cubes sug)	11.5	71.7	73.1	156			
Totals—Averages	95	315	205	615		418	109

One of the eggs may be exchanged for 3 Domino lumps or 2 heaping teaspoons of sugar which may be used in coffee or tea or on berries or grapefruit

Two eggs and glass of milk may be exchanged for one shredded wheat biscuit or five tablespoons of puffed rice with cream and part of above sugar

LUNCH

	PRO TEIN	FAT	CARBO HYDRATES	CAL- ORIES	URIC ACID GRAMS	PRO TEC TIVE	NONPRO TECTIVE
Vegetables cooked choice of any three							
Beets 2 h tbsp	66	07	21.2	29	trace	29	
Carrots 3 h tbsp	22	16	13.9	18		18	
Onions 1 whole	49	167	20.1	42		42	
String beans 2 h tbsp	20	61	4.7	13	0.006	13	
Spinach 2 h tbsp	86	38.1	10.7	57	0.072	57	
Asparagus 3 stalks about 120 grams	77	12	14.4	23	0.024	23	
Cauliflower 2 h tbsp	44	11	1.0	8	0.024	8	
Vegetables raw choice of any three							
May be prepared as salad with lemon juice							
Lettuce (¼ average head)	1.3		7.7	9	0.009	9	
Tomato (Group II med size)	9.8	37	32.8	46		46	
Celery (3 small stalks)	21	05	7.9	8	0.015	8	
Endive (4 stalks)	20		50	7	trace	7	
Water cress (3 h tbsp)	14	10	7.4	9.8	trace	9.8	
Cheese choice of one of the following							
Cottage							
Edam (Group III)	27	51	2	80	0.025	80	80
Swiss (average helping)							
Roquefort (about 20 grams)							
Two Saltines							
Bread one slice as in breakfast	11.3	3.6	65.3	80			80
Butter one pat 15 grams	0.6	118.6		119			119
Beverages							
One glass whole milk	29.8	81.8	45.1	157		157	
or							
One glass buttermilk	26.8	10.1	42.9	80		80	
Totals—Averages	90	260	200	550	0.025	300	305

Option Plain fresh fruit salad no sugar or dressing or with 2 tbsp French dressing or 1 tbsp dressing half mayonnaise and half whipped cream instead of Group I

DINNER OR SUPPER

	PRO TEIN	FAT	CARBO HYDRATES	CAL- ORIES	URIC ACID	PRO TEC TIVE	NONPRO TECTIVE
Soup—Vegetable, prepared without meat stock Except potatoes or lima beans, 4 oz, or	14 3		2 5	17			
Six oysters, raw No condiments except lemon juice	21 6	9 5	12 9	44	0 087		44
Meats, fish or fowl Choice of one only							
Beef, well done, sirloin, 1 slice, lean 100 grams	95 7	15 4		111	0 111		111
Mutton leg, 1 slice, 75 grams	76 9	157 6		234	0 078		234
Lamb chop, 1 chop, 100 grams	89	278 1		367	0 114		367
Fish { Perch Sole Any way except Trout fried Bass About 100 grams Cod	85 } Average	5 } Average	7 } Average	97 } Average	0 130 0 130 0 168 0 130 0 114		97
Chicken, roast, 1 slice, 100 grams	131 6	40 9	8 6	181	0 087		181
Vegetables, raw, choice of any three May be prepared as salad with lemon juice							
Lettuce	2 3		5 7	9 0	0 009	9 0	
Tomato	9 8	3 7	32 8	46		46 0	
Celery	2 1	0 5	5 9	8 0	0 015	8 0	
Endive	2 0		5 0	7 0	trace	7 0	
Water cress	1 4	1 0	7 4	9 8	trace	9 8	
Vegetables, cooked, choice of any three							
Beets, 2 h tbsp	6 6	0 7	21 2	29	trace	29	
Carrots, 3 h tbsp	2 2	1 6	13 9	18		18	
Onions, one whole	4 9	16 7	20 1	42		42	
String bean, 2 h tbsp	2 0	6 1	4 7	13	0 006	13	
Spinach, 2 h tbsp	8 6	38 1	10 7	57	0 072	57	
Asparagus, 3 stalks	7 7	1 2	14 4	23	0 024	23	
Cauliflower, 2 h tbsp	4 4	1 1	2 0	8	0 024	8	
Fresh fruit salad—no sugar or dressing, 4 heaping tbsp Fruits in season	2 0	8 0	120 0	130		130	
Bread, one slice, as in breakfast No butter	11 3	3 6	65 3	80			80
Beverages							
One glass whole milk or buttermilk	29 8 26 9	81 8 10 1	45 1 42 9	157 80		157 80	
Totals	175	290	310	775	0 114	430	225
Totals for entire three meals	360	865	715	1940	0 139	1180	760

THE BASIC DIET AS PRESENTED TO PATIENTS

Breakfast—

Choice of one of the following fruits

One orange, sliced or as orange juice (no sugar)

One half grapefruit (no sugar)

Strawberries, raspberries, blackberries (3 heaping tablespoonfuls, no sugar)

Two eggs, any form, except fried¹

One slice of thin whole wheat or white bread (toasted)

Butter, one pat (15 grams)

Beverages, one glass of whole milk² or one cup of coffee or tea ($\frac{1}{4}$ cup of milk, 1 tbsp cream, 2 cubes sugar)¹One of the eggs may be exchanged for three Domino lumps or two heaping teaspoons of sugar which may be used in coffee or tea or on berries or grapefruit²Two eggs and glass of milk may be exchanged for one shredded wheat biscuit or five tablespoons puffed rice with cream and part of above sugar

Lunch—

Vegetables, cooked 1 Choice of any three of the following
 Beets 2 heaping tbsp carrots 3 heaping tbsp onions one whole string beans
 3 heaping tbsp spinach 2 heaping tbsp, asparagus, 3 stalks (about 125
 grams) cauliflower, 2 heaping tbsp
 Vegetables raw Choice of any three of the following (may be prepared as salad
 with lemon juice)
 Lettuce $\frac{1}{4}$ average head 1 medium sized tomato 3 small stalks of celery, 4 stalks
 of endive 3 heaping tbsp water cress
 Cheese choice of one of the following
 Cottage Edam Swiss Roquefort (average helping about 20 grams) Two
 saltines
 One slice of white or whole wheat bread (toasted)
 Butter one pat (15 grams)
 Beverages one glass of whole milk or one glass of buttermilk

Dinner or Supper—

Soup vegetable prepared without meat stock except potatoes or lima beans
 (4 oz) or 6 oysters raw No condiments except lemon juice
 Meats fish or fowl Choice of one only
 Chicken 1 slice (100 grams)
 Lamb chop 1 chop (100 grams)
 Mutton leg 1 slice (70 grams)
 Beef sirloin, 1 slice lean (100 grams)
 Fish perch sole trout bass cod (any way except fried about 100 grams)
 Vegetables cooked Choice of any three
 Beets 2 heaping tbsp Carrots 3 heaping tbsp Onions one whole String
 beans 2 heaping tbsp Spinach 2 heaping tbsp Asparagus 3 stalks Cauli
 flower 2 heaping tbsp
 Vegetables raw Choice of any three of the following
 May be prepared as salad with lemon juice
 Lettuce $\frac{1}{4}$ average head One medium sized tomato 3 small stalks of celery
 4 stalks endive water cress 3 heaping tbsp
 Fresh fruit salad (no sugar or dressing) 4 heaping tbsp (Fruits in season)
 Bread one slice as in breakfast No butter
 Beverages One glass whole milk or one glass of buttermilk

*Avoid—*Preserved canned spiced salted smoked or corned meats or fish pork tongue
 goose duck turkey kidneys stews salmon mackerel herring shell fish, liver sweetbreads
 tripe and fat meats

Peppers garlic spices gravies Highly seasoned sauces mustard tobacco paprika
 horse radish parsnips

Nuts preserved sweetened syrupy fruits Pastry pies confections sugar jellies jams
 preserves ice cream pudding custard Hot rolls hot biscuits hot bread corn bread bran
 Meat extract soup and bouillons

All alcoholic or malt drinks

*General Comments and Directions—*Drink water freely between meals, a
 minimum of ten glasses per day

Meats and fish allowed only once every other day Eat only foods pre-
 scribed, none others allowed Be moderate in size of portions

Remember this diet is elastic and will be amended as the progress of the
 case indicates Progress will be determined by the general condition, weight
 and blood chemistry

Option Plain fresh fruit salad no sugar or dressing or 1 tablespoon dressing $\frac{3}{4}$
 mayonnaise $\frac{1}{2}$ whipped cream instead of cooked vegetables

SUMMARY

1 Chronic arthritis is becoming increasingly important to the medical profession because of its growing social and economic significance

2 The prognosis of chronic arthritis is good in a large percentage of cases, when treated by men who take a special and keen interest in the disease and are willing to treat it with great patience, zeal and perseverance

3 No single type of therapy is efficacious in the treatment of all forms of chronic arthritis. All the etiologic possibilities must be most carefully searched for and eradicated in so far as possible

4 The regulation of diet is of utmost importance in the treatment of chronic arthritis

5 Each patient should be placed, for a short period, at the beginning of treatment on a basic diet which is then regulated and adjusted to fill the individual requirements of the case. The regulation of the diet depends largely on such factors as the patient's general condition, weight, blood chemistry and clinical progress

6 The Basic Diet must have the following characteristics

- a Low caloric value
- b Low carbohydrate, protein and purin values
- c Adequate vitamin content
- d Elasticity
- e Availability

7 A Basic Diet for chronic arthritis is presented

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THE USE OF DRUGS IN THE TREATMENT OF ATROPHIC ARTHRITIS*

By A G LOENG, M.D., BOSTON MASS

IN A DISCUSSION of the use of drugs in the treatment of arthritis, one is impressed with the fact that most patients suffering from this disease have been the victims of drug treatment rather than the beneficiaries. Perhaps this is due to the lack of information regarding the etiology, pathogenesis and therapeutics of arthritis. One must also consider the fact that any chronic, painful disease usually places the patient at the mercy of analgesic therapy. Relief from pain is greatly appreciated by the patient and as a consequence analgesia has superseded the search for the etiology and curative treatment of the disease in the minds of many physicians.

Arthritis is one of the oldest diseases known. Sir Armand Ruffer reported lesions of hypertrophic and atrophic arthritis in the remains of Egyptian and Nubian mummies, dating from the period of about 8000 B.C. Despite the antiquity of the disease and its painful and debilitating nature very little progress has been made as regards its etiology, pathogenesis or treatment. For years arthritis was not distinguished from rheumatic fever and gonorrheal arthritis. This led to many systems of classification which were largely of a clinical descriptive nature and resulted in a confusion of terms which made it impossible to understand an article unless careful clinical histories were presented to explain the nomenclature used. The medical profession at large has considered chronic arthritis as an incurable disease which could be controlled somewhat by analgesic drugs. Empirical treatments of every description have been advocated by medical and nonmedical men, frequently leaving the patient a victim of neglect and quackery. Within the past two decades rheumatic fever and arthritis have received the serious study of a number of investigators who have gradually presented fundamental studies which form a foundation on which a more thorough understanding of these diseases can be based. As a result of these studies, a more rational therapeutics has evolved, which although far from attaining perfection, has shown the fallacy of certain measures and the value of those methods of treatment which are directed toward elimination of the cause of the disease and the restoration of the disturbed physiologic processes.

In order to discuss the rationale of the therapeutic measures which are used it is necessary to briefly review the clinical course of atrophic (including infectious) arthritis†. The role of foci of infection as etiologic factors was stressed by Billings¹ and the systemic nature of the disease has been pointed out by numerous writers.

From the Robt. B. Brigham Hospital

†At a meeting of the American Committee on Rheumatism in Philadelphia, March 17, 1928 chronic arthritis was divided into two main types: the atrophic and hypertrophic. The atrophic group includes the infectious arthritis.

The onset of atrophic arthritis may be acute or insidious. In those patients with an acute onset marked by a febrile reaction there is usually a focus of infection or a history of a recent infectious disease. The joints show the cardinal signs of inflammation and there may be muscle pains also. There is usually a mild leucocytosis. The chief complaint is pain and stiffness of the joints and a sense of extreme fatigue. The condition may subside within a few days to a few weeks or may pass into a chronic state. The pain is not always comparable to the degree of swelling, but usually there is direct relationship between the two. In the chronic state the disease may continue to be marked by continuous or intermittent swelling of the joints but without loss of weight or general debility. The soft tissues about the joints are swollen and there may be an accumulation of fluid within the joint. The joint space, cartilage and bone give a normal appearance. There is usually a mild secondary anemia, with or without a leucocytosis. However, if the patient becomes "atrophic" a general systemic reaction occurs marked by loss of weight, a mild anemia, a lowered systolic blood pressure and mild tachycardia, atrophy of the muscles (especially those about the affected parts) and atrophy and spindling of the shafts of the bones, with a softening and distortion of the ends. There is narrowing of the joint space and a loss of interarticular cartilage. The palms of the hands and soles of the feet are usually cold and sweat profusely. The nails become hard, brittle and ridged. Later they may drop off. The skin becomes dry, glistening and parchment-like, showing that the peripheral circulation has been greatly diminished. This can be verified by the use of the capillary microscope. The posture of the patient is poor. Gastrointestinal disturbances, especially constipation, are common. The affected joints usually become fixed in a flexed position due to the fact that the patient holds them in that position because it is the least painful. Later, distortion may take place, due to muscle spasm, which is so severe that dislocations occur. The basal metabolism is slightly lowered in most of these patients.² There is a mild anemia, frequently characterized by a low hemoglobin and a normal red cell count. Constipation is usually marked, associated with diarrhea, visceroptosis, etc. This résumé of the general condition is only an attempt to point out the major and most common signs and symptoms in order to more intelligently discuss the use of the various therapeutic measures employed.

THE DRUGS USED IN THE TREATMENT OF ARTHRITIS

The drugs used can best be classified according to their effect on the patient

1 Analgesics

{ Salicylates
Cinchophen
Acetanilid and phenacetin

2 Drugs used for their effect on the circulation

{ Digitalis
Nitrites
O Iodoxybenzoic acid

3 Drugs used for their specific action

{ Autogenous vaccines
Emetine

4 Drugs used for their nonspecific action

{ Foreign protein
Milk
Iodides

5 Tonics

{ Arsenic
Strychnine

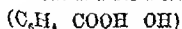
6 Drugs used for their nutritional value

{ Vitamins
Calcium

7 Laxatives

In the above classification, one notices that most of the analgesics also fall under the second heading. This is explained by the fact that most of them are also diaphoretic and therefore increase the peripheral circulation. However, this action is of short duration and cannot be depended upon.

I ANALGESICS

Salicylic Acid and Its Derivatives

Under this heading the salicylates undoubtedly rank first. (However Hanzlik³ says that cinchophen has an identical action.) The free acid is irritating causing nausea and vomiting when given internally. For this reason the salts are used. The acid has some antiseptic properties but the salts are practically devoid of this action.

Absorption —

Cutaneous—In fatty and alcoholic solutions, salicylic acid and its esters are readily absorbed from the intact skin (Hanzlik¹). Bourget⁴ found that lard or lanolin was the best vehicle for absorption.

Gastrointestinal—The soluble salts are slowly absorbed in the stomach while the insoluble salts pass into the intestine where they are rendered soluble before absorption occurs.

Distribution —

After absorption salicyl is found in practically every secretion fluid and organ of the body (Hanzlik²). Scott, Thoburn and Hanzlik⁵ compared the salicyl content of the blood and joint fluid of patients suffering from rheumatic fever. There was slightly more salicyl in the blood than in the joint fluid. Apparently there is no selectivity of salicyl for the inflamed joints.

Excretion —

Salicylates are excreted in the urine mainly as such (Hanzlik³). The rate of elimination depends upon the condition of the patient and the type of compound used. Hanzlik and his collaborators⁶ report a median duration of seventy eight hours in normal subjects, seventy two hours in rheumatic patients and of eighty hours in patients with miscellaneous conditions. In regard to the type of compound it was found that the salicyl derivatives require a much longer period for complete excretion than the sodium salt. In rheumatic fever the total excretion was found to be 15 to 20 per cent⁷ than in normal individuals. They believe this to be due to increased retention of the salicyl in the rheumatic,

Action —

Local —Salicylic acid is an irritant and dissolves the epithelial cells. This irritant action also prevents its use internally since it causes nausea, vomiting, and diarrhea. Sodium bicarbonate is prescribed with sodium salicylate to prevent the precipitation of free salicylic acid.

Emetic Action —Emesis can be produced with any of the salicylates, regardless of the mode of administration, if sufficient dosage is given since this action is due to a central effect as demonstrated by Eggleston and Hatcher.⁸

Kidney —Therapeutic and toxic doses of salicylates affect the kidney as shown by increased permeability to uric acid (and possibly other metabolites), the production of albuminuria, the changes in urinary output and renal function. Hanzlik and his collaborators^{9, 10, 11} have shown diminution in renal function and the production of albuminuria of renal origin in rheumatic and nonrheumatic patients following therapeutic doses of salicylates.

Circulation —All concentrations of salicylates tend to relax the blood vessels in perfused animals. In therapeutic doses the circulatory effects are probably secondary to the antipyretic and diaphoretic action which appears to be of central origin as is also the analgesic action.

Respiration —Respiration is not affected by ordinary doses but full therapeutic doses depress and quicken it.

Ears —The tinnitus aurium, characteristic of cinchonism, occurs after full therapeutic doses of salicylates. The susceptibility varies with the individual but this symptom is usually a good indication to stop administration of the drug.

Metabolism —(a) Nitrogen. The excretion of nitrogen is generally increased as shown by Denis and Means⁵¹ on normal subjects taking a known diet. The patients received up to 6.6 gm of salicylates daily and showed an increased excretion of nitrogen, also an increase in the phosphates and uric acid.

(b) Sulphur. Baumann and Herter¹² showed an increase in sulphate excretion. This was confirmed by Kumagawa.¹³

Diaphoresis Temperature and Heat Regulation —Sweating usually occurs after therapeutic doses of salicylates. It is usually more profuse in febrile conditions, sometimes becoming so severe as to produce a state of exhaustion. The mechanism is imperfectly understood but Hanzlik suggests that it is associated with the vasodilator action (central) which is so important a factor in its producing antipyresis.

Effect upon Immune Bodies —Swift^{14, 15} gave daily doses of sodium salicylate orally to rabbits that had been injected intravenously with living and dead *Streptococcus viridans* cultures and with washed sheep red blood cells. He found that the animals receiving the salicylate suffered a decrease in the formation of antibodies, agglutinin, hemolysin and complement fixation. Also he found that the rabbits receiving intravenous injection of antigens which had been previously treated in vitro with sodium salicylate showed lower antibody curves than did the rabbits receiving untreated antigen intravenously and salicylate gastrically.

Toxicity —

The symptoms are characterized by nausea, vertigo ringing in the ears headache, vomiting, and occasionally diarrhea

The postmortem changes consist of destruction of erythrocytes congestion of most viscera, necrosis of the spleen lymphoid elements and nephritis The lethal dose in man is about 1 gram per kilo

Idiosyncrasy —

The acetyl derivative provokes hypersensitive reactions more frequently than the other derivatives The reaction appears to be similar to allergic conditions and is marked by increased capillary permeability The symptoms are constriction and edema in the throat with dysphagia and salivation,¹⁰ congestion in the nose, swelling of the eyelids and edema of the pharynx^{17 18 19}

Salicyl edema has been described by Hanzlik and his collaborators,⁷ who believe it is due to disturbed renal function

Cinchophen

Chemically cinchophen or atophan is phenyleinchoninic acid Its formula is $[C_6H_5, C_6H_4, N(COOH)]$ It is quite insoluble but the sodium salt is soluble and nonirritant Its chief clinical derivative is the ethylester of methyl phenyleinchoninic acid $(CH_3, C_6H_4, N, C_6H_5, COOC_2H_5)$ It is known as "neocinchophen," "tolysin" and "novatophan" It is not readily soluble in water acids, or alkalies but is very soluble in the lipid solvents

Pharmacologically Hanzlik³ says that the action of cinchophen is exactly the same as that described for salicylates in so far as the cinchophen actions have been studied It is certain that its toxic and clinical actions are practically indistinguishable from the salicylates Therefore it is obviously unnecessary to repeat the information just detailed for the salicylates

Methods of Administration —

Oral—This is the chief route of administration of cinchophen and the salicylates since practically all of the compounds are readily absorbed when introduced into the gastrointestinal tract Nausea from gastric irritation can be reduced by administering sodium bicarbonate with the salicylates or cinchophen but the nausea due to large or repeated doses is central and therefore cannot be avoided by other routes of administration

Rectal—This method is sometimes advantageous when the patient is suffering from nausea due to other causes than the drug Heyn⁹ recommends it highly The first adult dose consists of 8 to 10 gm of sodium salicylate in 120 to 180 cc of plain or starch water If no symptoms of intolerance appear it may be repeated in twelve hours

Intravenous—There is no advantage to be gained by the intravenous administration of either salicylates or cinchophen Conversely this method does predispose the patient to reactions and according to Hanzlik³ can cause considerable harm to the heart and other organs and may cause collapse For this reason this method is mentioned only to point out its lack of value and possible danger

Clinical Considerations —

In administering salicylates or cinchophen to an arthritic patient, one can depend only upon their properties of analgesia and antipyresis, since there is no evidence to prove that these substances have any curative properties and there is considerable evidence to prove that they are devoid of any such action. Thus Swift²¹ showed that salicylates decrease the properties of the blood for specific antibody formation. Miller²² showed that the prophylactic administration of salicyl did not protect rabbits against arthritis produced by intravenous injection of hemolytic streptococci. Davis²³ found the rabbits with streptococcal arthritis that were treated with salicylates frequently died sooner than the controls. Swift and Boots²⁴ had a similar experience in that the salicylates appeared to produce an exacerbation of the infection and the cardiac lesions were not influenced. They concluded that the earlier death in the treated rabbits was due to the combined bacterial action on the kidney and the nephrotoxic effects of the salicylates.

These experiments and others led Hanzlik³ to conclude that "the antichemotic actions of cinchophen and salicyl are variable and undependable, and, when obtained they probably occur at the expense of circulatory depression."

It is not the purpose of this paper to discuss the action of these substances in rheumatic fever, however, there is a close analogy to be drawn.

In conclusion one may say that cinchophen and salicylates are efficient analgesics and antipyretics. In administering them to arthritic patients one can relieve the pain and muscle spasm, thereby permitting the patient to relax and rest, these are very important objectives to be obtained, but no permanent relief should be expected from such measures.

Anilin Derivatives

The anilin derivatives, acetanilid ($C_6H_5NHCH_2CO_2$), and phenacetin ($C_2H_5O C_6H_4NHCH_2CO$) are two of the most powerful analgesics and antipyretics known. However, they exhibit in a milder degree the toxic actions of anilin such as destruction of the red blood corpuscles, methemoglobinemia and the production of severe cardiac lesions especially of the conduction mechanism of the heart^{25, 26}

Phenacetin is less toxic than acetanilid and may be used occasionally in five or ten grain doses without injury, but in a chronic condition such as arthritis it should only be prescribed when other analgesics have become intolerable.

Morphine and Codeine

One is seldom justified in administering morphine to an arthritic patient. It is a dangerous procedure to start since the patient, even in the acute stage, usually suffers pain for a sufficient period of time to develop a morphine habit.

Codeine is not so prone to produce a habit but is usually not as efficient as salicylates or cinchophen in relieving the pain. Therefore, one should use narcotics in arthritis only under very unusual circumstances, thereby avoiding the possibility of starting a habit which will only add to the patient's discomfort and physical injury.

II DRUGS USED FOR THEIR EFFECT ON THE CIRCULATION

As has previously been mentioned the circulation of the patient with arthritis is very noticeably disturbed. This is a prominent clinical symptom as shown by a mild hypotension and tachycardia with mild to severe trophic disturbances due to depression of the peripheral capillary circulation. Boas and Rifkin⁷ and McClae⁸ have pointed out the incidence of cardiac lesions in arthritis deformans. The work of Rowntree and Adson⁹ has shown that surgical release of the sympathetic control to the part is followed by noticeable symptomatic improvement.

Digitalis

The knowledge of the pharmacology and uses of digitalis is so universally disseminated that it would be superfluous to repeat it here. Pemberton¹⁰ recommends the administration of daily doses of 9 to 15 minims of the tincture to improve the circulation. The rational of this treatment is not clear, since there is no evidence to show that the heart is ordinarily involved in arthritis. It is definitely known that digitalis does not affect the normal heart unless given in toxic doses. Therefore its value in the treatment of arthritis not complicated with heart disease is questionable. Where definite cardiac signs or symptoms are present larger doses should be given.

Nitrites

Nitrites reduce the tone of the arterial musculature, thereby producing an immediate fall of blood pressure due to vasodilatation. This effect is particularly noticed in the vessels of the skin. The action appears soon (one to five minutes) after administration and disappears within five to fifteen minutes.

Pierce and Pemberton¹¹ administered doses of $\frac{1}{4}$ to $\frac{1}{2}$ grains of erythroltetranitrate to a selected group of patients. They obtained a "favorable influence" in 12 out of 32 cases treated.

Possibly this is a step in the right direction but due to the fact that nitrites produce methemoglobinemia if given over an extended period and also the fact that the vasodilatation is brought about at the expense of a lowered blood pressure it would appear that nitrites are not the ideal vasodilators for the treatment of arthritis.

O Iodoxybenzoic Acid "Amidoxyyl Benzoate" (N N R)

Chemically this substance has the formula ($C_6H_4COOHIO$). The ammonium salt was officially called amidoxyyl benzoate by the Council on Pharmacy and Chemistry. It was introduced as a therapeutic agent for arthritis by Young and Youmans¹² in 1926.

Pharmacology —

Local — The free acid and its salts are nonirritant to the unbroken skin, but when injected hypodermically, a red area appears associated with a stinging burning sensation. This persists for a short time and is not followed by a slough. When applied to the mucous membranes it acts as an oxidizing agent and is effective in the treatment of Vincent's angina. The pure ammonium salt will not injure the mucous membranes. It is antiseptic especially for staphylococci and streptococci and the colon typhoid group.

Heart and Circulation —

Heart — In the perfused heart Loevenhart and Eyester³² found that sodium iodoxybenzoate produces rigor. They say "The power to cause the heart to develop rigor is apparently due to the power to oxidize something in the heart muscle." The substance would not replace molecular oxygen in the perfused heart.

Circulation — Experimentally, Loevenhart and Grove³³ found an occasional slight fall in blood pressure, with an increase in cardiac output. They made the very interesting observation that "Cardioplethysmographic tracings showed that the fall of blood pressure is not due to any cardiac effect of the drug. Perfusion of the isolated intestines proved that the salt does not act directly on the vessel wall" and concluded that the fall in blood pressure is due to depression of the vasomotor center. This is further substantiated by observations on the capillary flow of the nail bed which is greatly increased as is also the lymph flow. The author found that the lymph flow from the thoracic duct of a fasting dog was increased four to five times by the injection of 4 cc. of a 1 per cent solution of the ammonium salt.

Blood —

Sodium iodoxybenzoate oxidizes hemoglobin to oxyhemoglobin (Loevenhart and Grove).

Effect on Immunologic Reactions —

Arkin³⁴ found that sodium iodoxybenzoate stimulates phagocytosis of streptococci and staphylococci by human leucocytes. He attributed this to an activation of the opsonin by the "oxygen containing substance," since a substance which liberates oxygen readily stimulates phagocytosis.

Hektoen³⁵ found that this substance stimulates the production of specific hemolysins in dogs. Arkin³⁶ repeated this work on rabbits and obtained the same results. He also obtained an increase in the production of specific agglutinins in rabbits immunized with killed typhoid bacilli. He suggests that these results are due to an acceleration of the oxidation in the tissues which are the site of antibody formation.

Effect Upon Allergic Reactions —

Amberg and Knox³⁷ studied the effect of sodium iodoxybenzoate in allergic reactions and found that while it did not influence the general allergic reaction it did prevent the local reaction such as one sees in the tuberculin test.

It was also shown that intravenous injection of this compound inhibits the inflammatory reaction produced by mustard oil. They, therefore, concluded that the action is an antiinflammatory reaction or an antichemosis. Arkin³⁸ showed that tuberculin is oxidized by the drug *in vitro*.

Respiration —

Therapeutic doses do not affect the respiration but in toxic amounts a temporary apnea is produced.

Fate and Excretion —

The salts of o-iodoxybenzoic acid are reduced to o-iodobenzoic acid and excreted largely in the urine as o-iodohippuric acid. These experiments were

performed on patients and although the results were not satisfactory from a quantitative standpoint they coincide with the work of Novello, Miriam and Sherwin¹⁸ who fed o iodobenzoic acid to dogs and found it to be excreted in the urine partly unchanged but mostly as o iodo hippuric acid. The distribution in the tissues has not been determined. It is important to state here that o iodoxybenzoic acid *does not break down in the body or test tube to yield iodides or salicylates*

Toxicity —

The toxicity of the pure salt depends more upon the rate of injection than upon the amount given. In experimental animals death is usually of a respiratory nature but may be circulatory due to heart rigor.

Postmortem findings are largely negative. The most consistent finding is congestion of the lung bases. Chronic poisoning has never been produced in animals nor has it been seen in patients.

A death has been reported by Manace¹⁹ and I have been informed of another death but in this latter instance the drug was submitted to a pharmacologist who found that "it contained some very toxic ingredient aside from the ammonium salt of iodoxybenzoic acid."

Reactions —

In an acute or subacute condition the administration of the drug will usually provoke a febrile reaction which lasts for from one half to two hours. In chronic patients the administration is marked by a stinging sensation of the mucosa of the nose and mouth, the face becomes flushed, the patient usually salivates and may become nauseated. There may be a sensation of substernal oppression. A tingling sensation is noticed in the extremities. This reaction usually subsides within ten to forty five minutes after the administration of the drug. In some patients the nausea persists for a longer period, vomiting may occur and occasionally a mild diarrhea. The author has never experienced a fatality or a reaction of alarming proportions which could be attributed to the drug.

Repeated examinations of patients treated over a long period of time have failed to show evidence of nephritis.

Administration —

(a) *Intravenous* — This method was recommended by Young and Loumans²¹ in their first report and has proved to give the most satisfactory results. One gram of the ammonium salt is dissolved in 100 cc of sterile salt solution and given by gravity method over a period of ten to fifteen minutes. To avoid too rapid flow into the vein it is well to use a small needle (about 22 gauge) and elevate the gravity set about four feet above the patient's arm. If this method is followed *using a pure product and rubber tubing that has been thoroughly cleansed* alarming reactions will not occur.

(b) *Rectal* — Smith²⁰ reported favorable results when the drug was given in the form of a retention enema. He used 15 to 2 gm in 200 cc of normal saline. A cleansing enema should precede the administration of the drug by two hours. This method is advantageous and usually effective in patients who are not suitable for intravenous therapy.

(c) *Oral*—The drug has been given orally in the form of the calcium salt in capsules. The insolubility of the calcium salt decreases the irritant action and obviates nausea due to the ammonium ion which is liberated from the ammonium salts. The dose consists of 1.5 to 2 gm. The results obtained are variable. In some patients quite satisfactory results occur, but in a larger percentage it is unsatisfactory.

Clinical Considerations—

The clinical use of the salts of o-iodoxybenzoic acid is of recent origin and therefore one should be conservative in attempting to evaluate this drug in the treatment of arthritis. However, since the first report by Young and Youmans in 1926, fifteen papers reporting 589 cases have been published. The average improvement reported shows that 66.3 per cent showed marked to moderate improvement, while 23.7 per cent showed slight or no improvement. Of this group, most of the patients were classified as "chronic," "infectious" or "proliferative." The remainder were acute infectious or gonorrheal with a few cases of rheumatic fever and a few hypertrophic arthritides. These figures include the report of Stein and Taube⁴¹ who treated 102 cases (about one-sixth of the patients reported) with no improvement. This report merits especial consideration since it is the only published account of complete failure to obtain improvement with this drug. It is interesting to note that the report included 10 cases of acute infectious arthritis and 2 cases of rheumatic fever. Only the rheumatic fever patients were confined to bed. The authors say "Any improvement, two weeks after the administration of the drug, we consider not to be due to the drug." Obviously this is not a fair estimate of the value of the drug since as Neighbors⁴² remarked in commenting on this same paper "While it is true that relief of symptoms often begins during treatment, it is a repeated observation that improvement in joint symptoms may not be apparent until a few weeks have elapsed, and an improvement in general health may be still later manifested." Furthermore, it is not consistent with past clinical experience to expect improvement in acute infectious arthritis who are not confined to bed. In the chronic patients, undoubtedly other methods must be combined with the drug treatment in order to obtain improvement. It is pertinent to repeat here the statement made by Young and Youmans³¹ in the original report on this drug "We believe that it will never be possible with a single drug to cure a patient with arthritis who has developed permanent deformity and crippling, and that the proper treatment is a combination of methods, including the use of prophylactic and curative orthopedic procedures, the removal of foci of infection and general hygienic measures as well as drug therapy."

In his first report on this drug following the work of Young and Youmans, Smith⁴⁰ pointed out the chemical relationship between o-iodoxybenzoic acid and salicylic acid, i.e., that one is the o-iodoxy- and the other the o-hydroxy-derivative of benzoic acid. He suggested that their actions might be very similar, and stressed the analgesic action of o-iodoxybenzoate. This idea has been accepted by some observers and is quoted by Bartlett⁴³ and by Pemberton³⁰ who says that the drug is "a glorified salicylate." The error of this conclusion is readily demonstrated by the fact that o-iodoxybenzoate acts in

the body by virtue of its available oxygen attached to the iodine. This was demonstrated by Loevenhart and Grove³³ and Loevenhart and Eyester³ and has since been confirmed by me when I recovered the drug from the urine in the form of o-iodo hippuric acid. Salicylic acid has no available oxygen in its molecule. Further evidence of the difference in action of the two compounds is shown by the fact that o-iodoxybenzoate increases phagocytosis, the formation of specific hemolysins and agglutinins and definitely depresses the sympathetics. Salicylic acid inhibits the formation of specific antibodies and does not affect the sympathetics in ordinary dosage. The salicylates act chiefly by virtue of their analgesic and antipyretic properties whereas o-iodoxybenzoates act by virtue of their oxidizing properties which bring about a depression of the sympathetics thereby increasing the peripheral capillary and lymph flow. They are also antinflammatory agents by virtue of their antihemetic action.³⁷

In a later report Smith⁴⁴ concluded that patients who are suffering from gastrointestinal disturbances did not respond to treatment with o-iodoxybenzoate. The author cannot agree with these findings since most chronic arthritides are constipated and a large percentage have responded to this treatment. Certainly patients are encountered who are not improved by the use of this drug, but in my experience these patients have not shown a higher percentage of gastrointestinal disturbance than usual. At present there is no plausible explanation which will cover the entire group.

III. DRUGS USED FOR THEIR SPECIFIC ACTION

Autogenous Vaccines

The use of vaccines made from cultures of diseased tonsils, teeth, etc. has been practiced for several years. Frequently good results are reported. However, it is very difficult to ascertain to what extent the vaccine acts by virtue of its specific influence and to what extent it may be due to a foreign protein reaction. There is also the difficulty of definitely proving that the organism removed from the tonsils, teeth, etc. was responsible for the arthritis. On the whole the results have not been sufficiently encouraging to warrant general use. Further development of the work of Small⁴ and Cecil, Nicholls and Stainsby⁴⁵ may contribute more valuable biologic agents than we have at present.

The vaccine is administered intramuscularly, usually in increasing doses. Beginning with a dose of 10,000,000 organisms it is gradually increased to 75,000,000 or more. Febrile reactions are not uncommon and may be of therapeutic value. The patients frequently complain of increased joint pain during the treatment.

Emetine

Ely⁴⁷ and his collaborators have advanced the idea that hypertrophic arthritis is due to *Entameba histolytica* which enters the body through root infections of the teeth and passes from there to the marrow spaces. Barrow and Armstrong⁴⁸ report finding the organism frequently in the stools of arthritis patients.

As a consequence ipccac and its alkaloids have been used by mouth and hypodermically Up to the present time no work has been done to confirm the findings of Ely and therefore it is not possible to evaluate this treatment

IV DRUGS USED FOR THEIR NONSPECIFIC ACTION

Under this heading come typhoid vaccine, "mixed cultures," milk, and some colloidal compounds which are capable of producing a febrile reaction All of these substances are dependent upon the production of a febrile reaction for their therapeutic results Typhoid vaccine is one of the most commonly used agents It is injected intravenously in doses varying from 15,000,000 to 175,000,000 dead typhoid organisms Within one-half hour to two or three hours the patient experiences a chill and fever associated with headache, malaise, nausea, and sometimes vomiting This is followed by profuse sweating and a gradual return of the temperature to normal The duration of the reaction as well as its severity depends upon the number of dead organisms in the dose given It frequently lasts twenty-four to thirty-six hours after which the patient may experience a pronounced relief from pain and stiffness These treatments are usually given at three to seven day intervals, sometimes with increasing doses The effect produced from an immunologic viewpoint and its value is difficult to determine However, it is definitely known that during the period of the "chill" when the temperature is rising, there is a profound depression in the rate and volume of the capillary circulation to the skin At this time the patient is the most uncomfortable and may complain of increased joint pain Following this period when sweating begins, the capillary circulation to the periphery is greatly increased in rate and volume and this is associated with relief from pain This effect persists for about twenty-four hours after the fever has subsided and the joint improvement persists for a longer time In some instances this is sufficient to restore the physiologic function of the joint circulation until the body recovers sufficiently to carry on the normal circulation

One should bear in mind that this procedure is "shock therapy" and in large doses as 125,000,000 dead organisms there is danger of severe reactions and even death Histologically, amyloid disease may be produced in experimental animals I have seen thrombosis of the vessels of the feet and legs so severe as to cause gangrene and necessitate a bilateral amputation, following large intravenous doses of dead typhoid bacilli Severe atrophic arthritides are frequently unable to withstand shock therapy

Iodides

Since the action of iodine is not definitely known its use (largely as potassium iodide) must be considered to be empirical Pemberton³⁰ suggests that its action is due to its influence on the thyroid gland It is usually described as an "alterative" Regardless of what action the iodides may have, they have proved to be valuable therapeutic adjuncts, when combined with other measures

When given by mouth they are expectorants and may produce emesis They are readily soluble and are absorbed rapidly For this reason *there is nothing to be gained by intravenous injections which may be followed by severe edema*

Endocrine Substances

Since there is no definite information on which to base an opinion as to the role played by the endocrines in arthritis, it is difficult to evaluate or explain the use of the endocrine extracts in this condition

Thyroid —

Thyroid in the form of whole dried gland has been used extensively in doses of from 5 to 15 gr daily until the pulse rate or nervous condition of the patient indicates that it should be discontinued. Despite the fact that the atrophic arthritic may have a slightly lowered basal metabolism and responds to thyroid medication, one cannot conclude that he is suffering from hypothyroidism. As a matter of fact he is more frequently the *hyperthyroid* type of individual. For this reason one cannot determine how desiccated thyroid exerts its beneficial action. Improvement may be obtained without changing the basal metabolism. Some patients are definitely intolerant to thyroid medication therefore it is advisable to begin with small doses ($\frac{1}{2}$ to 1 gram, t i d), which may be gradually increased.

Ovarian Extracts —

Since arthritis frequently manifests itself during the menopause considerable attention has been given to the relation of the ovaries to arthritis in women. At the present time no definite information is at hand and opinion varies greatly as regards the therapeutic value of ovarian extracts in arthritis occurring at this time.

V TONICS

Since patients with atrophic arthritis usually suffer from loss of weight, anorexia, severe fatigue and anemia, tonics play an important part in the treatment.

Arsenic

Arsenic has been used for many years either as Fowler's solution by mouth or injected as sodium cacodylate. In either form the benefit gained probably is the result of its action on the blood regeneration elements and upon metabolism.

Iron

The anemia which accompanies arthritis indicates the use of iron. However, one is frequently disappointed in the results obtained. Usually the anemia does not improve until there is definite improvement in the arthritic condition.

Strychnine

This is the only tonic known in the true sense of the word and is valuable in attempting to restore the muscle tone and prevent atrophy. It is prescribed in many ways, but the well known "I Q and S" mixture appears to give good results and has the added advantage of containing iron.

VI DRUGS USED FOR THEIR NUTRITIONAL VALUE

Vitamins

The great advances in nutritional chemistry have thrown much light on the action of the vitamins and their influence on health and disease.

Since arthritis is a disease involving the bones and therefore very probably the calcium metabolism, *Vitamins A and D*, in the form of cod liver oil, have been extensively used. The beneficial results are difficult to evaluate, but on the whole one is impressed with the fact that patients receiving cod liver oil maintain their appetite and body weight better than those who do not receive it.

Vitamin B —

The gastrointestinal disturbances so frequently encountered in arthritis indicate the value of Vitamin B. This has been especially shown by the work of Fletcher and Graham⁴⁹ who found a definite loss of tonicity and loss of haustral markings in the colon in atrophic arthritis. Vitamin B may be administered in the form of yeast, or better, the yeast extract described by Wakeman and Osborn,⁵⁰ known commercially as "Harris Yeast." The dose is six to nine tablets daily. The yeast extracts obviate the gas which frequently accompanies administration of the yeast itself.

Vitamin C —

No definite value can be ascribed to Vitamin C in the treatment of arthritis, but since the citrous fruits are recommended because of their value as basic salts, this vitamin is usually consumed in large amounts. Certainly it can do no harm and it may be beneficial as a "blood builder" as shown by its effect in scurvy.

Calcium —

The patient with arthritis frequently shows a low urinary P_H and a high total titrable acidity. This indicates a disturbed acid-base balance and probably a loss of calcium. Since the urinary reaction can be changed by the administration of basic salts, calcium in the form of its soluble salts (as calcium lactate) is of special value. It is also well to prescribe milk, custards, etc. Calcium administration should be forced until the P_H of the urine is neutral or slightly alkaline.

VII LAXATIVES

Most patients with arthritis suffer from constipation and give a history of requiring frequent laxatives. One should attempt to correct the constipation by posture, exercises, diet and proper hygienic measures. However, one frequently finds that some stimulus is necessary to produce a bowel movement.

Of the milder substances, mineral oil and agar are very suitable. For a laxative cascara or phenolphthalein are quite effective and can be given in diminishing doses as the other measures begin to take effect. The greatest problem is to educate the patient to rely on his habits and not on a laxative for his daily evacuations.

DISCUSSION

In presenting this paper it is not with the intention of promulgating the idea that arthritis may be overcome by simply administering a large number of drugs. No other disease requires more painstaking study and individual attention for successful treatment. Each patient is an individual problem worthy of careful study and thought. The disturbed physiology is a predom-

nating feature. The physician is confronted with the problem of discovering the cause, the present and past exciting factors and what physiologic processes are disturbed. He may then attempt to remove the cause and correct or restore to normal the disturbed physiology. Some of this treatment can be carried out by the intelligent use of drugs. Unfortunately the patient with arthritis has been too frequently the victim of analgesic remedies with no attempt to remove the cause or correct his present disturbed processes. As a result, valuable therapeutic agents have fallen into disrepute and patients have become hopeless invalids who might have been restored to an active life.

When drug treatment is instituted it should be done with the definite purpose of correcting some phase of the patient's illness. In order to do this, one must know his patient clinically and understand the pharmacology of the drug to be used. Drugs used in this manner in conjunction with other methods, as indicated by the condition of the patient, will be of distinct value and contribute toward the improvement which can be obtained in a large percentage of these patients when properly treated.

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THE TREATMENT OF CHRONIC 'INFECTIOUS' ARTHRITIS BY SYMPATHETIC GANGLIONECTOMY AND TRUNK RESECTION*

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ASIDE from removal of foci of infection the major and relatively more successful forms of treatment for chronic 'infectious' arthritis are all directed toward (1) increasing the circulation of the joint (2) increasing the temperature and thus the metabolism of the joint and (3) increasing oxidation of articular tissue. These are supposed to be the beneficial results of physical means of treatment such as heat and cold, dry and moist applications, diathermy, massage, exercises and heliotherapy and also of certain 'medical' procedures, such as fever therapy and administration of preparations of thyroid gland.

The treatment of certain cases of chronic arthritis by resection of sympathetic ganglia and trunks was instituted by Rowntree and Adson in the hope that this surgical measure might produce an optimal degree of articular circulation in certain joints at least. The details of the technique, the rationale of the procedure and the results in the first seventeen cases have been given in a series of papers; this paper will give but a survey of the subject.

UNDERLYING PRINCIPLES

Any superiority in results that may come from resection of sympathetic ganglia and trunks in certain well selected cases of chronic infectious arthritis may lie solely in the fact that by this procedure the desired beneficent state is maintained over a protracted time, possibly permanently, instead of intermittently for only a few minutes or hours at a time.

The general principle of resection of sympathetic ganglia and trunks is to cut and remove the sympathetic ganglia and their rami that contain vasoconstrictor fibers to the vessels of the extremities, thereby increasing the circulation and temperature of the joints of the extremities and probably increasing tissue oxidation in them.

Data are not at hand to support the idea that resection of sympathetic ganglia and trunks corrects a primary 'neurogenic' cause for chronic polyarthritis. But in the light of our studies we feel that the neurogenic theory merits further investigation. In 1889 Spender graphically described the tangled web of strange neurotic things that comprised at least part of the picture of chronic arthritis. Even before this the old humoral theory of the causation of arthritis had begun to be supplanted by the conceptions of Remak, Chareot, Trousseau, and others in favor of a primary neural genesis. Garrod, although supporting the theory of dystrophy, believed that the associated nervous

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phenomena were secondary rather than primary. After the theory of infection was launched by Schuller, the theory that the disease was neurogenic lost ground until certain clinicians tried to reconcile conflicting views and suggested that infectious trophoneurosis was present.

Several investigators have reported the finding of certain pathologic changes in various portions of the central nervous system in arthritis. In many instances there are strikingly present such clinical phenomena as altered vasomotor tonus, hypotension, pigmentation, cyanosis of the skin over the joints, clamminess, and increased sweating, particularly of the extremities. No one has definitely determined whether they result from some primary and noninfectious disturbance of the central or sympathetic nervous system, or from secondary disturbances arising from the effect on the nervous system of some toxic, bacterial or metabolic insult. The present consensus of opinion is that they are secondary neurogenic phenomena, the sympathetic nervous system participating in a general systemic disturbance, which might well be of infectious origin. Whatever the source, there is evidence that resection of sympathetic ganglia and trunks may restore, to a certain extent, some of these neurogenic alterations, chiefly, however, in the most distal joints of the extremities.

CLASSIFICATION OF CASES

Suitable for Operation—We have limited use of the operation to cases of chronic infectious arthritis. This term is in general synonymous with the terms atrophic, proliferative, rheumatoid, and periarticular arthritis. It is held to be in contradistinction to osteoarthritis, hypertrophic arthritis and degenerative arthritis. However, we use the term without assuming that our opinion on etiology is final, but we use it in line with the clinical classification of Hench, which is based on presumptive cause, and that is used on the arthritic service of this clinic (Tables I and II).

Operation is indicated probably in only a small percentage of cases of chronic "infectious" arthritis. Satisfactory results have been obtained frequently in cases in which there have been changes in soft tissue of the joints of the hands and the feet. No benefit has been obtained by certain of our patients. These experiences are the basis of the six major criteria which at present are the guide in selection of cases deemed suitable for this operation. These are as follows:

- 1 The "arthritis" should be chiefly periarticular or synovial (capsular) with little, if any, bony alterations (destruction or hypertrophy) except atrophy.

If marked bony alterations have occurred, resection of sympathetic ganglia and trunks is not expected to accomplish any definite restoration of the lost bone and cartilage. Furthermore, when the bony changes are marked, similarly extensive changes may have occurred in the soft tissue, with injury to the vascular supply, probably irreparable to a large degree.

- 2 Patients preferably should demonstrate some of the alterations in vasomotor tonus, shown objectively by cold, clammy, sweating hands and feet, reduction of blood pressure (approximately below 110 to 115 systolic), and subjectively by intermittent numbness and tingling.

When the extremities are already warm the caliber of the vessels is presumed to be sufficient or nearly so, and resection of sympathetic ganglia and trunks would not be expected to increase materially the articular circulation. When arteriosclerosis is present adequate vasodilatation may be impossible of attainment even with operation. In the presence of either of these conditions the vasomotor index (Brown) will be low.

3 Vasomotor alterations must be capable of correction or of overcorrection, by means of release from control of the sympathetic apparatus. The possibility of such correction can be demonstrated by determining the "vasomotor index",

TABLE I

TENTATIVE CLINICAL CLASSIFICATION OF DISTURBANCES OF THE JOINT BASED ON PRESUMPTIVE ETIOLOGY

-
- | | |
|---|---|
| 1 | Infectious arthritis |
| | Known to be specific infectious for example |
| | Tuberculous |
| | Gonorrheal |
| | Pneumococcus |
| | Typhoid |
| | Syphilitic (spirochetal not arthropathy) |
| | Staphylococcal (septic) |
| | Probably specific infectious (with toxins) |
| | Rheumatic fever (streptococci or their toxins) |
| | Arthritis with amebic colitis (amebic or secondary streptococci or their toxins) rare |
| | With ulcerative colitis (secondary streptococci or their toxins) rare |
| | With certain skin diseases (especially psoriasis) |
| | Nonspecific (chronic infectious type) streptococcal |
| | Articular nonspecific infectious arthritis |
| | Nonarticular localization (myositis fibrositis lumbago) |
| 2 | Traumatic arthritis |
| | Extrinsic trauma (generally acute) (1) articular (traumatic, baseball fingers), and (2) nonarticular (nails, knees and housemaid's knees sprains strains) |
| | Intrinsic trauma (generally chronic) postural arthritis |
| | static arthritis of obesity |
| 3 | Senescent arthritis chief sites |
| | Fingers (Heberden's nodes) |
| | Hips (morbus coxae senilis) |
| | Hypertrophic spine of the elderly, cervical frequently lumbar most common |
| | Knees (often in combination with static arthritis of obesity) |
| 4 | Gouty arthritis |
| | Acute (recurring with complete remissions) |
| | Chronic (progressive with residual deformity) |
| 5 | Arthropathy |
| | Secondary to lesions of the central nervous system (syringomyelia, Charcot's disease) |
| | Secondary to certain lung conditions (pulmonary osteoarthropathy) |
-

that is, by obtaining definitely higher cutaneous temperature than mouth temperature after typhoid vaccine has been given intravenously. The temperature of the joints of the extremities may or may not be identical with that of the skin over them, but the temperature of the latter serves as an available index of elevation of temperature from vasomotor dilatation.

The "vasomotor index" is determined as follows: fifty million triple typhoid" bacilli (typhoid, paratyphoid A and B) are given intravenously. The

oral temperature may rise but 2°C , whereas the cutaneous temperature over the joints of the extremities may increase 10 to 14°C , a ratio of 1 to 5 or 1 to 7 . The best results seem to occur in cases in which the ratio is high and in which the increase in temperature is more than 5°C .

4 The patient should be preferably less than thirty-five years of age, and not more than the age of forty-five years.

Chronic "infectious" arthritis generally appears before the age of thirty-five or forty years. The majority of cases of arthritis in patients who are more than the age of forty-five years belong to the type here called chronic senescent (degenerative, hypertrophic) arthritis. Furthermore, after the age of forty-five years, the presence of arteriosclerosis, preventing adequate vasodilatation, may act as a contraindication.

5 The arthritis should be progressive, and the main disability should be confined to the extremities, particularly to the hands and feet. If the arthritis is not progressive, continuation of the therapeutic program already established may accomplish satisfactory results. If it does not, and if the degree of disability is great, operation may be permissible in carefully chosen cases.

TABLE II
CLASSIFICATION OF CHRONIC ARTHRITIS IN MOST COMMON USE

AUTHOR	FIRST TERMINOLOGY*	SECOND TERMINOLOGY*	TERMINOLOGY BASED ON
Goldthwaite Nicholls and Richardson	Atrophic Proliferative	Hypertrophic Degenerative	Pathologic changes (bone) Pathologic changes (soft tissue and bone)
English Fisher Various authors	Rheumatoid Synovial Periarticular	Osteoarthritis Chondro osseous Hypertrophic Destructive	Clinical data Anatomic site Roentgenogram

*The terms in this column are in general synonymous.

6 A reasonable period, probably at least six to twelve months, of intensive, not haphazard treatment by the more established, less radical procedures, should be allowed before resection of sympathetic ganglia and trunks should be considered. However, rapidity of progression or stress of economic circumstances may necessitate consideration of earlier surgical measures.

There will be wide variation in what is considered to constitute a reasonable period of treatment, and more particularly as to what is meant by adequate previous treatment. No one patient probably ever had all the various forms of treatment deemed by various observers to be adequate. For the individual clinician, decision on this point will rest on the failure, first of that program, whatever it is, which has in general been for him the most successful, and with the additional failure of such other promising, but by him less consistently used, measures that he feels are worthy of trial.

Operation of Limited Application—On a few occasions we have been led, by stress of a patient's progressive physical and economic disability and his desire for operation, to perform resection of sympathetic ganglia and trunks in the presence of definite, bony and cartilaginous alteration. In general the results have been disappointing as to the degree of reduction of symptoms, inflammation and articular stiffness obtained. Of course bony alterations already

present were unaffected. In some pain was not greatly alleviated nor progression of the condition markedly influenced. In some the marked improvement noted in the early postoperative course was not maintained. This was not the universal outcome, however. In at least the following case relief of pain was obtained in joints in which the arthritic process had gone on to include definite hypertrophic and destructive changes, with considerable deformity.

REPORT OF CASE

A woman, aged twenty six years began to have arthritis in the hands especially but also in the feet. Twelve years before she came to the clinic she had had two periods of relative quiescence respectively of two and two and a half years' duration. The last exacerbation had started in February 1929 after which time the arthritis had been markedly progressive. The arms, elbows and hands especially were very painful. The arthritis progressed steadily in spite of treatment which included extraction of several teeth, apendectomy many prolonged periods of intensive physiotherapy, several courses of administration of typhoid vaccine intravenously, a diet high in calories and vitamins and directed against constipation, several weeks treatment by colonic irrigation and a diet high in Vitamin B and two series of treatments by roentgen rays. The tonsils had been removed prior to the onset of the arthritis. There was marked ulnar deviation of both hands and contraction deformities of the fingers. The hands especially were cold clammy and painful and there was flexion deformity of the elbows without complete ankylosis. The shoulders were mobile but painful. The knees and feet were involved to a less degree than the hands.

Roentgenograms gave evidence of marked destructive and hypertrophic changes particularly in the fingers, wrists, elbows and feet with periarticular changes in the shoulders and knees. The vascular index for the right index finger was 4.

Because of the progressive pain resection of sympathetic ganglia and trunks was suggested, in spite of the definite bony alterations. It was done in the cervical region with the hope of relieving pain and possibly checking the progress of the disease particularly in the joints, in which thus far changes were in soft tissue only.

Although the deformity and bony changes present at the time of operation are in the main unaltered at least to date (six months postoperatively) the patient has experienced marked analgesic effects from the cervical operation (Table III) and in addition has less stiffness and more muscular strength in the hands. Because of this she requested that the lumbar ganglia and trunks be resected also and this has just been done.

TABLE III

DECREASE OF PAIN IN JOINTS FOLLOWING CERVICOTHORACIC SYMPATHETIC GANGLIOMECTOMY PERFORMED TO RELIEVE PAIN (IN SPITE OF THE PRESENCE OF SOME BONY CHANGES)

JOINTS	BEFORE OPERATION GRADE		FORTY DAYS AFTER OPERATION (GRADE)			FIVE MONTHS AFTER OPERATION GRADE		
	AT REST	ON VOL- UNTARY MOTION	AT REST	ON VOL- UNTARY MOTION	ON FORCED MOTION	AT REST	ON VOL- UNTARY MOTION	ON FORCED MOTION
Right hand	~	3	1	1	2	0	0	1
Left hand	1	2	0	0	1	0	0	1
Right elbow	3	3	0	1	1	0	0	1
Left elbow	2	2	0	0	1 2	0	0	1
Right shoulder	1	1	0	0	1	0	0	0
Left shoulder	1	1	0	0	1	0	0	0
Right wrist	2	3	0	1	2	0	0	1
Left wrist	2	2	0	1	2	0	0	1

Pain graded on the basis of 0 to 4

Operation not Indicated—There are certain types of chronic infectious arthritis in which resection of sympathetic ganglia and trunks is not advocated.

Some of these types have been mentioned, namely, cases of chronic infectious arthritis with definite bony involvement, cases in which the extremities are already warm and the caliber of the arteries is maximal or nearly maximal, and cases in which arteriosclerosis probably would prevent vasodilatation. Another type is chronic infectious arthritis involving the spinal column. Surgical technique by which resection of sympathetic ganglia and trunks can be applied in arthritis of this structure is not available. Still another type for which the operation is not advised is chronic infectious arthritis involving the hips and shoulders. When the process is localized in the elbows or knees resection of sympathetic ganglia and trunks may afford more chance of relief than it does for involvement of the shoulders and hips. However, limited relief has been obtained in disease of these regions, as compared with that of the hands and feet, possibly because the temperature of these joints is so much less elevated by the operation. Furthermore, the operation is not advocated for chronic arthritis of the types generally considered noninfectious—the forms designated as degenerative arthritis, osteoarthritis, and hypertrophic arthritis. These forms are generally more localized and less polyarticular in nature.

TECHNIC

Since the technique of resection of lumbar sympathetic ganglia and trunks has been previously described by Brown and Adson and remains unchanged, it will not be outlined here. The technique of the cervicothoracic operation has been modified somewhat from that of previous descriptions. It was noted that by the technique formerly used, the manifestations of Horner's syndrome were not always equal on both sides. This suggested that gray, postganglionic fibers were entering the carotid plexus, therefore Adson has changed the operative procedure by resecting the vertebral portion of the first rib instead of the second. The lateral portion of the transverse process is also resected with the rib in order to facilitate the exposure. This permits entrance into the mediastinum opposite the first thoracic sympathetic ganglion. The first thoracic ganglion is then dissected free and the trunk is resected just above or below the second thoracic ganglion, depending on whether the second is situated high or low with relation to the second thoracic nerve. If the second thoracic ganglion is not removed, the first thoracic nerve is carefully dissected from the intervertebral foramen to its juncture with the brachial plexus in order to interrupt thoroughly all gray rami that may ascend from the second thoracic ganglion. After elevating the thoracic ganglia and trunk, the entire lower cervical ganglion is resected with the chain. This alteration in the technique has produced complete and permanent bilateral Horner's syndrome, and, it is hoped, has included all vasoconstrictor fibers to the upper extremities.

EFFECTS

Physiologic—The recognized physiologic effects of the operation are apparently without deleterious results, if one views as justifiable by-products, the Horner's syndrome, dryness of the skin and absence of sweating. This statement is made after an experience at the clinic of five years since beginning such operations, first on patients with spastic paraplegia. Then it was applied suc-

cessively in cases of Raynaud's disease, thromboangitis obliterans, scleroderma and arthritis. The physiologic effects may be summarized as follows:

1. Marked vasodilatation. This was illustrated in injections performed by Horton and Craig. The vasodilatation apparently affects chiefly the moderate and small arteries and arterioles. There is probably no vasodilatation of the capillaries. At least the capillaries of the nailfold are constricted after the operation, but the rate of flow is augmented.

TABLE IV

STUDIES OF SURFACE TEMPERATURE IN CHRONIC INFECTIOUS ARTHRITIS BEFORE AND AFTER LUMBAR SYMPATHETIC GANGLIONECTOMY

CASE	FEET				
	BEFORE OPERATION DEGREES C		ABOUT 2 TO 3 WEEKS AFTER OPERATION DEGREES C		AVERAGE INCREASE DEGREES C
	RIGHT	LEFT	RIGHT	LEFT	
2	23.8	23.2	28.4	28.6	5.0
4	25.3	26.2	30.2	31.0	4.8
5	25.7	27.2	33.5	34.0	7.3
6	23.8	25.0	34.6	35.0	10.4
7	27.8	28.4	33.8	34.6	6.1
8	28.5	27.6	32.2	32.0	4.0
9	25.4	25.0	33.4	32.9	7.9
10	28.1	25.6	33.9	33.1	6.6
11	24.1	24.7	33.1	32.8	8.5
12	22.4	23.0	34.5	34.0	11.5
14	20.2	20.3	33.8	34.0	13.7
15	26.2	25.9	33.6	33.9	7.7
16	25.5	26.8	33.0	32.9	7.3
17	26.2	25.7	34.0	34.4	8.4

*Average increase in temperature 7.8 C

TABLE V

STUDIES OF SURFACE TEMPERATURE IN CHRONIC INFECTIOUS ARTHRITIS BEFORE AND AFTER CERVICAL THORACIC SYMPATHETIC GANGLIONECTOMY

CASE	HANDS*				
	BEFORE OPERATION, DEGREES C		ABOUT 2 TO 3 WEEKS AFTER OPERATION DEGREES C		AVERAGE INCREASE DEGREES C
	RIGHT	LEFT	RIGHT	LEFT	
3	29.0	29.3	34.5	34.7	5.4
13	28.4	28.2	33.8	34.1	5.6

Average increase in temperature 5.5 C

TABLE VI

STUDIES OF SURFACE TEMPERATURE IN CHRONIC INFECTIOUS ARTHRITIS BEFORE AND AFTER LUMBAR AND THORACIC SYMPATHETIC GANGLIONECTOMY

CASE	BEFORE OPERATION DEGREES C				AFTER OPERATION DEGREES C.				AVERAGE IN CREASE DEGREES C	
	FEET		HANDS		FEET		HANDS			
	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	FEET	HANDS
1	25.9	24.1	23.5	24.1	34.5	34.0	33.9	33.3	9.3	9.8

2. Definite increase of temperature of the extremities as determined by studies of surface temperature with the Stewart Kegerreis calorimeter and Sheard electrothermocouple. In the hands and feet the temperature may in

crease 5 to 13° C, in the elbows and knees generally not more than 2 to 4°, and in the shoulders and thighs, approximately only 1 to 2 or 3° (Tables IV, V and VI)

3 Abolition of reflex sweating If areas of sweating persist in the upper extremities after the operation they have been considered evidence of incomplete resection. An operation anatomically correct in technique is followed by complete abolition of reflex sweating.

4 Loss of pilomotor reflex

5 The reaction of cardiac acceleration not markedly disturbed. There is apparently no marked alteration of cardiac rhythm or rate.

6 A Horner's syndrome which is permanent if the operation is anatomically correct. It does not give rise to significant alteration of vision, however.

7 Some minor atrophy of shoulder muscles in the region of the wound after cervicothoracic operation.

8 No objective atrophy of muscles of the lower extremities after the lumbar operation. Instead they may enlarge and again become fusiform.

9 Relief of pain of variable degree. In the extremities the degree and rapidity of analgesia may be gratifying. Relief of pain precedes the other beneficial effects that may result, such as diminution of redness, tenderness, swelling, and stiffness. On awakening from the anesthetic several patients experienced unusual, even complete, relief of pain. Increased warmth of the extremities occurs as the immediate result of the operation. In some cases the relief of pain was maintained, in others it was not. In some cases relief of pain has been only partial at first, and has become complete within eight weeks to six months after operation. In other cases, relief has never been complete and in some cases there has been no diminution of pain. With further experience in selecting and managing cases it is hoped that relief of pain can be expected to be maintained.

A satisfactory explanation of analgesia by resection of sympathetic ganglia and trunks cannot be given. Craig has commented on it briefly thus:

"There are two chief theories concerning this subject. (1) that the sympathetic nerves carry sensory fibers, and (2) that the sympathetic nervous system regulates in some manner the threshold of pain. The last theory seems to be the most logical. Here at the clinic we have not been so fortunate as to obtain 100 per cent good results in our operations on the sympathetic nervous system (for other conditions as well as for arthritis) for indefinite pain. If operation on the sympathetic nervous system is attempted in every case of indefinite pain, then the procedure will fall into disrepute."

Clinical—To date bilateral resection of cervicothoracic sympathetic ganglia and trunks only, has been performed at the clinic in two cases, bilateral resection of lumbar sympathetic ganglia and trunks only, in twenty-five cases, and both procedures in three cases. The results in those cases in which operation has been performed most recently will not be considered here. The preliminary results in the first seventeen cases have been reported elsewhere and need not be reviewed here. The patients, however, can be considered to have fallen into three groups.

1 Those who have obtained partial to almost complete relief from the manifestations of their arthritis, and in whom the relief to date has been maintained or progressive

2 Those who have obtained rather marked relief for a few weeks after operation but in whom there have been partial or complete relapses

3 Those who have obtained negligible relief

To aid in justifying a major operative procedure such as resection of sympathetic ganglia and trunks one would hope that at least primary beneficial results would be evident within three weeks to three months after operation or that the improvement would be maintained and progressive

In some cases there has been reduction of pain, indeed complete absence of pain, immediately after operation, with a slower reduction of redness, swelling and stiffness and perhaps no reduction of deformity. Because the analgesia is maintained, however, it seems justifiable to consider such results as definitely successful

When there is improvement after operation, for weeks or months, if the process then returns in the joints the vasomotor control of which has been released the procedure cannot be considered successful. Reinfection from other affected joints may be the cause of these exacerbations, but if the operative procedure cannot prevent this it cannot be considered a success

Certain other operative procedures for arthritis advocated by others from time to time (for example resection of the colon) have resulted in temporary success. It has been argued that the results came from the postoperative period of rest and the sharp metabolic stimulation of an operation, and that the specific operation itself was of no value. In attempting to evaluate resection of sympathetic ganglia and trunks dispassionately, we must remember that the factors of three or four weeks of rest after operation and the stimulation of the defensive forces of the body by the operation are possible factors in producing relief. Further experience and longer postoperative observation must be had before the degree and field of usefulness of the operation can be determined

Flothow and Spurling have both reported satisfactory results in five cases of chronic polyarthritis

SUPPLEMENTARY TREATMENT

In certain cases in which rather definite relief of pain resulted from the operation patients were inclined to overexercise the joints. This may prevent reduction of swelling, stiffness and redness. Patients must be prevented from causing too great trauma to the joints. Exercise should be extended with more careful attention to the reaction, not of pain but of other signs of inflammation

When a reasonable period of time has passed for judging the results of the operation itself, other rational procedures in treatment should be resumed. Complete removal of foci of infection presumably will have been done before operation was considered. Continuation of careful massage, muscular training exercises, reduction of deformity and protection of weakened joints by mechanical means are all indicated. Application of heat to the extremities is also rational, for although maximal dilatation presumably is accomplished in the

most distal joints of the extremities, it will be remembered that the increase in temperature in the less distal joints of the extremities is not nearly so great

CONCLUSIONS

Resection of sympathetic ganglia and trunks is not applicable to all forms of arthritis, nor is it useful in all stages and degrees of chronic infectious arthritis. In this experimental stage it is our impression that it is of definite value in certain carefully selected cases. It seems to be a justifiable procedure in these selected cases when all other reasonable measures have failed. Used properly, but not delayed too long, it may, by maintaining increase in temperature, circulation, and perhaps metabolism of the more distal joints of the extremities, induce a stage of compensation in the arthritic disability that is not capable of production otherwise. Our final opinion regarding the proper selection of cases for the operation, and its value in these cases, cannot yet be expressed.

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PAIN OF NERVE ROOT ORIGIN IN HYPERTROPHIC OSTEARTHTRITIS OF THE SPINE AS A CONFUSING FACTOR IN DIAGNOSIS

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OSTEARTHTRITIS of the spine may be the cause of pain and sensory disturbances of spinal root distribution, either from the meningeal reaction secondary to the osteoarthritic process or from pressure in narrowed canals. The radicular syndrome in hypertrophic osteoarthritis of the spine, described elsewhere, was based on the study of 70 patients at the University of California Medical School.*¹ Adequate descriptions of a root type of disturbance occurring in diseases of the spinal vertebrae and nerves other than in hypertrophic osteoarthritis may be found in systems of medicine² or in standard textbooks. These will not concern us at this time.

When one considers the anatomic distribution of the spinal nerve roots and the fact that the skin of the entire body, except part of the face and the anterior third of the head, contains sensory receptors for the spinal roots, it becomes obvious at once that, depending on the level of the pathologic process in the vertebrae, symptoms at the periphery will be manifested as painful or unpleasant sensations in areas that are also common sites for the reference of pain from various diseased viscera (Figs 1 to 10). It is not surprising, therefore, that a case history analysis revealed the variety of diagnoses shown in Table I.

Clinical History—Sufferers from hypertrophic osteoarthritis of the spine described the root areas of their chief symptoms with topographic accuracy. The extent of the painful area was accurately demarcated regardless of the region involved and was usually bilateral. In the past history, one could obtain the story of involvement of areas not complained of at the time. These, too, the patient demarcated with precision. The history of long standing revealed a story of involvement of various areas over a number of years and if a record listing the subjective areas according to the roots involved was made, one could anticipate the vertebrae that would show the pathologic process on the x-ray examination. Certainly the sharp demarcation and accurate localization of the painful areas, with involvement of the entire cutaneous distribution of the particular roots was a constant finding and in contrast to the usual vague localization and incomplete root zone distribution of the pain of the classical viscerosensory reflex.

Clinical Course Mode of Onset Duration and Description of Pain—Although the root pain of osteoarthritis might appear abruptly, the onset of the pain was not sudden in the sense of it constituting a seizure as one encounters in abdominal colics or in angina pectoris. The pain lasted for a variable length

of time from a few minutes to hours, and might disappear as quickly as it appeared. More often the cessation of pain was gradual or tapered off if a comfortable position was found.

The pain was generally bothersome and nagging and was usually described as an aching, soreness or as a paresthesia. Certain movements of the spinal column which the patient described by such phrases as rising after a sitting position, raising the head on awakening, getting out of bed, walking, lifting, sitting in one place for any length of time, a change of position, and the acts

TABLE I

VERTEBRAE AFFECTED	THE CHIEF COMPLAINT OR DIAGNOSIS	THE NUMBER OF DIAGNOSES OC- CURRING IN 70 PATIENTS
C2-3	Headache	8
	Thyroid disease	1
C4 or 5	Painful shoulders	
	Painful arms	
C4 or 5 with	Sore neck	
C6 and 7	Neuritis	
	Neuralgia	
	Arthritis of shoulder	
	Stiff neck	18
	Fibrositis or myositis	3
D1 to 3	Heart disease	
	Angina pectoris	
	Heart neurosis	50
	Pulmonary and pleural disease	2
	Painful breast tumor (size of pea)	1
D6 to 9	Stomach trouble	14
	Peptic ulcer	6
	Patients who first visited a gastro- enterologist for ulcer	3
		4
	Gall bladder disease	
D10 to 12	Lower abdominal pain	15
	Chronic appendicitis	4
	Pelvic disease	4
	Varices of broad ligament (laparot- omy was not performed)	1
L1 to 4	Neuralgia paresthetica	6
L4-5, and Sacral	Spondylia	23

The multiplicity of diagnoses is accounted for by the fact that the same patient was seen in various special clinics because of a preponderance of symptoms varying from one area to another at different visits. Such shifting of the chief complaint in the same patient from time to time is in itself suggestive of root pain in an individual who is suffering from diffuse involvement of the spine in the osteoarthritic process.

of coughing, sneezing and straining, caused the pain to appear or to become aggravated. The acts of coughing, sneezing, and straining in the production or aggravation of symptoms was very helpful in the diagnosis of the condition. Pain at night which awakened the patient or prevented sleep and was relieved by a change of position such as by "turning over" was commonly noted. In the far advanced, widely distributed vertebral process, the inability to sit up in bed from a supine position without producing pain and the necessity of rolling out of bed on one side or the other were quite characteristic.

Even though the pain in our cases was never severe enough to require opiates, sometimes, in the aggravated forms, it prevented or interfered with work more by virtue of its constant exacerbations following movements of the spine than from its severity. In the extreme forms and to a variable degree in the lesser forms, the sufferer had pain over the various root zones from morning to night, at rest, or in sleep. The pain appeared in one region or another when the head was raised from the pillow in the morning and disappeared after the patient had been up and about for a while only to return when active movements were again begun. Only temporary relief was obtained by resting in a chair, for on prolonged sitting the pain returned. Inter sleep was broken by the appearance of symptoms which necessitated a change of position for relief.

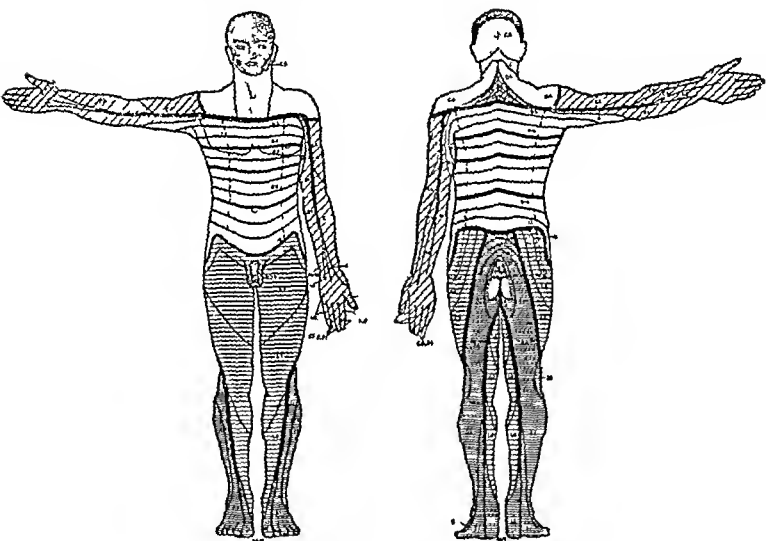


Fig 1—Radicular sensory innervation (Mayer J. A. M. A. 1918)

But ordinarily, during the daytime the patient was aware only of a disagreeable sensation. He was uncomfortable and nothing more. While seated, during the taking of the history he would tell the examiner that he had pain and would then proceed to outline it and to describe its character. Other than the patient assuming some particular posture for comfort such as leaning toward one side or the other, there were no outward signs to disclose the presence of his pain.

Often, as when the precordium was the seat of the chief complaint worry and fear over the constant discomfort might be the chief reasons for the visit to the physician. In such instances when the patient was asked whether he would have consulted a physician if the disorder was strictly confined to the right side he might reply "No I would pay no attention to it then, because

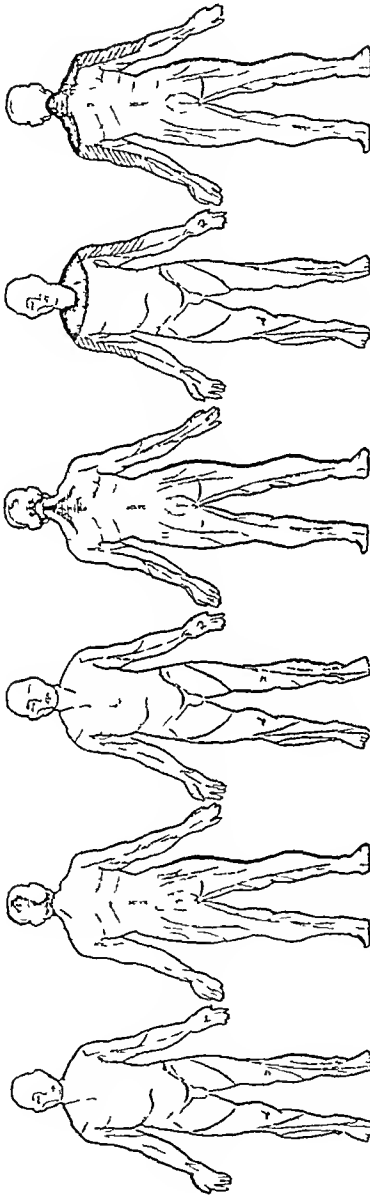


Fig 2 —Radicular distribution cervical 1, 2 and 3 occipital and vertex headache

Fig 3 —Radicular distribution cervical 1, 2 and 3 cervical 5 to 7 occipital and vertex "headache and back of the neck, pain

Fig 4 —Radicular distribution cervical 4 painful shoulders cervical 5 to 7 back of the neck, pain

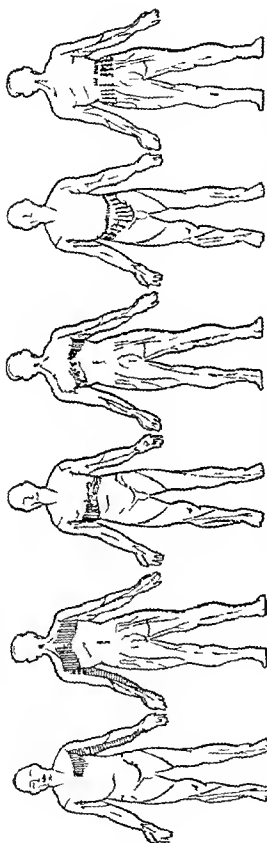


Fig 5—Radicular distribution dorsal to 5 pseudo angina pectoris

Fig 6—Radicular distribution dorsal 6 to 9 area of referred gall bladder pain on the right Gastric disturbances and pancreatitis.

Fig 10—Radicular distribution dorsal 10 belt like pains as in tabes dorsalis 11 1° on the right area of referred pain from acute appendicitis and on the left area of referred pain from adnexal disease

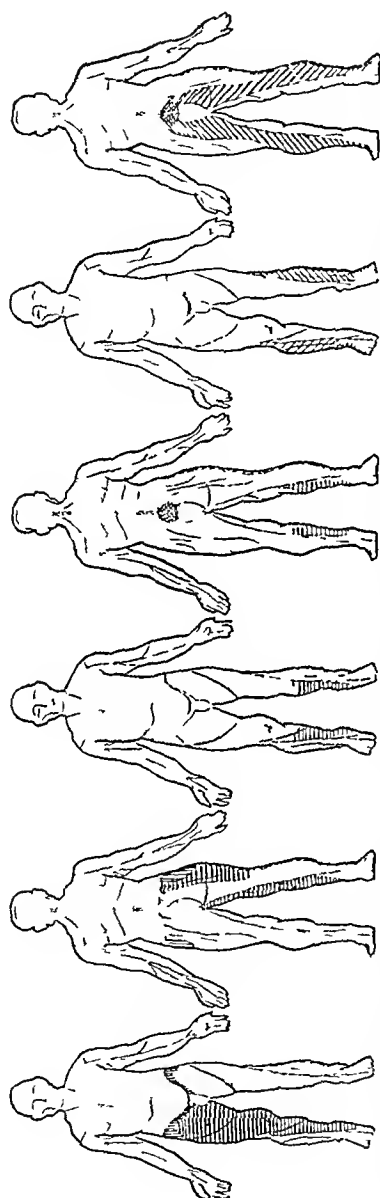


Fig 8—Radicular distribution lumbar 1 2 3 4 meralgia paresthetica hip-joint and knee-joint disease

Fig 9—Radicular distribution lumbar 4, 5 and sacral 1 over the sacrum, lumbar 4, inner side of legs

Fig 10—Radicular distribution lumbar 4 5 and sacral 1 over the sacrum with radiation over sacral 1 and 2, commonly spoken of as sciatica

the heart is not on that side.' During the manipulations of the spine, in the course of the physical examination certain movements of the spine would reproduce symptoms. Unless the patient called attention to the pain elicited during the manipulation the examiner would not ordinarily be aware of its presence because of the lack of outward signs to disclose the condition.

From time to time the site of the predominant symptoms shifted. At one examination the precordium occupied the patient's attention and at another the small of the back and the front of the abdomen or the area over the gall bladder and the back across the shoulder blades. Again it was chiefly occipital and vertex headache or shoulder neuritis and pain between the shoulder blades. The occurrence of pain, often many times in the twenty-four hours depended on the nature of the patient's activities. The duration of the pain did not seem to bear any relation to the frequency of the onset or the disappearance of symptoms. The painful areas were similar in their symptomatology and their response to remedial measures and they varied only in their regional location and intensity. The wearing of a corset or light brace strapping the back and heat and massage were measures that gave relief. Many of the patients preferred a hard bed, obtaining most rest when lying flat on the back and the placing of boards under the mattress sometimes solved the problem of broken sleep at night. Because of the variable zonal symptomatology the patient was apt to be ailing over long periods of time and to become a chronic complainer. In the clinic his chances of becoming labeled a neurotic were very great.

The Physical Examination—The physical examination revealed evidences of hypertrophic osteoarthritic processes in the peripheral joints as well as in the spine although the former were not invariably present. The usual findings consisted of Heberden's nodes on the fingers, or grating or crackling in the knee joints, shoulder joints or in the neck with variable degrees of restriction in the mobility of the affected joints. The spine generally showed restricted mobility of various degrees. The normal dorsal anterior bowing may have become exaggerated or flattened out in its upper or lower part. Sensory alterations to light touch as tested with the cotton tuft on a wooden applicator, and sensory disturbances such as hyperalgesia or hypalgesia to pinching could be demonstrated. The roentgen ray findings have been described in detail elsewhere.¹

Treatment—Temporary relief of pain was offered by the use of the coal tar derivatives and other analgesics. Salicylic acid, phenobarbital and codeine were helpful. Main reliance was placed on physical therapeutic measures such as baking, heat and massage and the immobilization of the spine in a properly fitted corset or a Taylor brace. Heat alone was found to be the most satisfactory agent for the milder cases. Vasodilators such as nitrites were without effect on the pain. Recently Swann⁴ has reviewed the subject of treatment and has suggested means of preventing the deformities associated with hypertrophic osteoarthrosis of the spine.

Conclusion—Osteoarthrosis of the spine is a common cause of pain of spinal root distribution. The syndrome of root pain in osteoarthrosis of the spine and its means of clinical description has been described.

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841 PACIFIC MUTUAL BUILDING

CHRONIC ARTHRITIS ITS CLASSIFICATION, ETIOLOGY AND PATHOLOGY WITH AN OUTLINE OF ITS RATIONAL TREATMENT*

BY LEONARD W ELY, M D, SAN FRANCISCO

A JOINT usually consists of a closed cavity, bounded by the articular cartilage, usually hyaline, and by a connective tissue membrane, the synovial membrane. These two structures meet at the articular margin of the end of the bone, and the structure of one seems to merge into that of the other, so that no exact termination can be made out between the two. In other words, the synovial membrane is not continued over the surface of the articular cartilage. The perichondrium is nonexistent in the normal joint after fetal life.

The articular cartilage consists of encapsulated cells, and of a hyaline basement substance. It contains no blood vessels, and is therefore not subject to inflammation. There is therefore no such thing as a chondritis. Strictly speaking the same may be said of an osteitis. The bone simply reacts to changes of its contained marrow, hence the absurdity of such terms as osteochondritis. The synovial membrane is the only tissue of a joint which can be subject to an inflammation. It is the active tissue of the joint. A synovitis therefore is an arthritis, and an arthritis is a synovitis. The marrow in the vicinity may or may not be inflamed.

Arthritis is usually divided into acute and chronic. The division is a convenient one but by no means exact. The line between the two is often not clear, a case which begins with all the characteristics of an acute arthritis may subside into a chronic state, and an essentially chronic arthritis may at any time exhibit acute manifestations.

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Chronic arthritis has been variously classified. The confusion in the classifications is so great that a student is often puzzled to understand an author's meaning. Again some of the classifications are so involved that it is almost impossible to remember them. Arthritis deformans means one thing in Germany, another in England and several things in America. In point of fact it means a deforming inflammation of a joint and any arthritis may be that. Osteoarthritis to one is hypertrophic arthritis to another, degenerative arthritis to a third and arthritis deformans to a fourth.

In all the history of medicine two methods of advance have been followed, but rarely side by side. One usually crowds out the other. They may be called the deductive and inductive, the philosophic and the experimental or the speculative and the investigative. The one on the basis of a few observations attempts to arrive at a conclusion by a process of abstract reasoning, and backs up the conclusion by extravagant claims of cures; the other accumulates a mass of facts and reaches its conclusions with relatively little reasoning. The first is easy, rapid, frequently profitable, and almost invariably sterile. It is the method of the past, and has been generally discarded except in diseases of the joints. Here it is too often followed. A knowledge of the pathologic anatomy of the joints and of the reaction of their tissues to injury and disease is usually considered superfluous. It is no more superfluous here than in any other branch of medicine. No one who does not possess it deserves an audience.

We have learned much on the subject of chronic arthritis during the past fifty years—little before that and nothing except from a patient accumulation of facts. Our experience should teach us that here as elsewhere in medicine we can expect little from ratiocination. What follows is based upon (1) clinical experience, (2) laboratory study of the pathology of arthritis, (3) experimental work on animals to test the truth or falsity of a theory.

CLASSIFICATION OF CHRONIC ARTHRITIS

The customary procedure is first to describe cases of known etiology under their own headings, tuberculous, syphilitic, etc. and then to classify the cases of unknown etiology according to some feature, clinical or pathologic, which the observer deems important. This is unscientific, confusing, and subject to frequent change. All cases of chronic arthritis fall into two classes or groups, which are as different as black is from white, and which can almost always be distinguished by clinical investigation aided by the x-rays. They can always be identified when the material is examined in the laboratory. They have been called by many names.* Nichols and Richardson's nomenclature probably comes nearest the truth.¹ Until many obscure points have been cleared up I prefer to call them Type 1 and Type 2.

THE TWO GREAT TYPES OF CHRONIC ARTHRITIS

First Type—This includes all the infectious arthritides, e.g., tuberculous, syphilitic, and also those cases whose exact etiology is not yet known and

A tuberculous joint in the old days was a fungous or strumous joint or a caries alca

which are called by various names, e g, rheumatoid, proliferative, atrophic Reading teaches us that these latter form a slowly dwindling group Tuberculosis, syphilis and others formerly belonged to it, perhaps under the caption of chronic "rheumatism"

Second Type—The cause of this type has never been determined The disease forms a clear-cut entity, and has been identified under various names, senile rheumatism, arthritis deformans (German), osteoarthritis (English), hypertrophic arthritis (Goldthwait), degenerative arthritis (Nichols and Richardson) etc When it occurs in the fingers it is known as Heberden's nodes, and is often mistaken for gout In the hip it is sometimes called morbus coxae senilis

THE FIRST GREAT TYPE OF CHRONIC ARTHRITIS

The members of this group enjoy a common pathology It follows therefore that their symptomatology and their radiologic characteristics are the same So much alike are they indeed, that, if the patient were covered with a sheet, exposing one joint for examination, we should be completely at sea The diagnosis is usually made from the history, and from a general examination of the patient A positive diagnosis can be made only by a demonstration of the causal organism In some of them, as in the arthritis caused by the tubercle bacillus, the *oidium coccidioides*, or the pus cocci, this is simple, and is a routine procedure In others, such as syphilis, it is much more difficult, and is rarely done If an arthritis is clinically syphilitic, we almost invariably rely upon the therapeutic test In a gonococcic arthritis the organism may be recovered from the joint on one day, and not on the next In a patient with an acute gonorrhea, whose knee suddenly swells and becomes exquisitely painful, we usually make the diagnosis with or without the recovery of the gonococcus There must be an ability in the synovial membrane to conquer infections, for gonococci in the synovial membrane have not the same significance as they have in the heart valves Indeed, there is such a power in the synovial membrane, for gonococcic joints often recover completely with or without rational treatment

This group includes also many cases whose exact etiology is still in doubt Until the causal organism or organisms shall have been recovered from them with reasonable frequency scientific scepticism is justified The reasons why I believe that they are all infectious are

- 1 They enjoy a pathology and a symptomatology similar to that of the known infectious arthritides If a piece of the synovial membrane be submitted to a microscopic examination, the pathologist reports chronic inflammation, tuberculous or nontuberculous as the case may be

- 2 Various investigators, notably Poynton and Prime, by diligent search have recovered infectious organisms from some of these obscure cases Others have sought for them in vain In this connection it must be remembered that in some infectious arthritides bacteria are fed into the synovial membrane intermittently from a distant focus, as are the gonococci in a urethral infection

*I have heard heated discussions as to whether there was any such thing as syphilitic arthritis In Albutt's *System of Medicine*, published about twenty-five years ago the statement is made that gonorrheal arthritis is caused by a reflex irritation of the urethral mucous membrane

If we do not find them we infer that they have been present, from the characteristic changes which they have left behind them in the joint tissues as we recognize a safe breaker from his finger prints on the safe or a woodchuck from his characteristic burrow

With the second type of arthritis bacteria have nothing to do and can be present only as an accident. Indeed in this second type of arthritis the joint seems to be practically immune from bacterial invasion. I do not remember ever to have heard of a so called second type joint being infected after an operation.

3 Every case of arthritis of this type whose cause is known tuberculous syphilitic gonococcic typhoid pneumococcic etc. is due to infection from a distant focus. Reasoning by analogy we conclude that other cases of unknown cause but of similar pathology are due to the same thing.

4 Many cases of intractable arthritis recover promptly after the removal of infected tonsils or after the cure of an infection in the deep urethra. To base a method of cure on such an argument as this is unjustifiable but to employ it as confirmation of other evidence is justifiable.

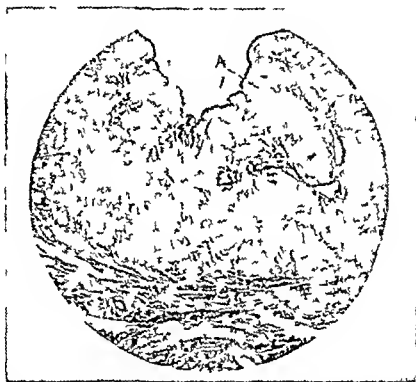


Fig 1—Tuberculosis of the synovial membrane with thickening infiltration and villous proliferation. This is the rapid severe form with practically no effort at encapsulation. Areas of necrosis at A.

PATHOLOGY OF TYPE I ARTHRITIS

The prime pathologic feature of this type of arthritis is as Nichols and Richardson first pointed out, a proliferative inflammation in the synovial membrane (Fig 1). To this may or may not be added a proliferative inflammation in the bone marrow in the immediate vicinity of the joint. This inflammation may start in either tissue and spread to the other. Certain easily understandable changes follow not only in the marrow and in the synovial membrane, but also in the bone and in the cartilage. Let us first consider those cases in which the inflammation starts in the joint tissues themselves that is, in the synovial membrane.

The synovial membrane becomes thickened infiltrated and villous. Sometimes the villous proliferation becomes extreme and the villi form masses (Fig 2). Usually an exudate is poured out into the joint cavity serous bloody fibrinous flocculent or purulent as the case may be. The fluid, to

killing it, gaining the joint either through it, or at its circumference. As soon as the synovial membrane is involved, it reacts as described above.

When much fluid accumulates in the joint cavity, it may rupture the capsule, and may then make its way to the surface, this, as is well known, is most likely in tuberculous and in suppurative infections.

SYMPTOMATOLOGY OF TYPE I ARTHRITIS

The symptoms are those of inflammation in any organ, pain, swelling, and interference with function. To these in the more active cases, redness, and increase of local temperature may be added. The pain is usually greater when the bone is involved than in the purely synovial cases. Sensitiveness to pressure is common. The swelling is in the soft parts. Bony proliferation

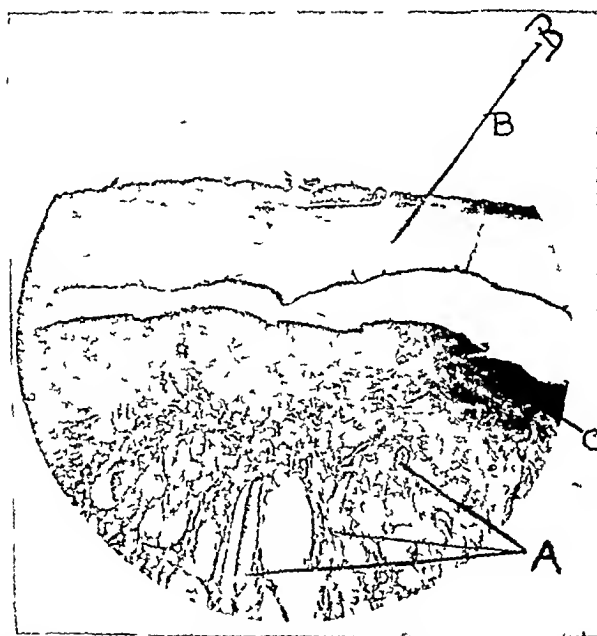


Fig 5—Tuberculosis of the marrow (C) directly under the cartilage (B). The cartilage has been sequestered and the bone trabeculae in the tuberculous area have practically disappeared. Bone trabeculae at A.

is absent in this type of arthritis. Flexion deformities are the rule, though superextension is often seen in the joints of the fingers. Limitation of motion is the rule, varying from a slight restriction at the extremes, to a complete ankylosis. Muscular spasm is common.

Constitutional symptoms may be present or absent, depending upon the nature of the infection, and upon its severity. Fever accompanies some, especially in their acute exacerbations. It is conspicuous by its absence in tuberculosis, when unmixed with a secondary infection. One does not expect any constitutional symptoms with a tuberculous joint, unless the patient has an active tuberculous lesion in some vital organ. Much the same may be said for syphilis, though here, as elsewhere in the body, this protean disease is wont to defy any rules laid down for it.

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gether with the thickening of the capsule, causes the joint to swell. In some cases the original proliferation may be small in amount, and free fluid cannot

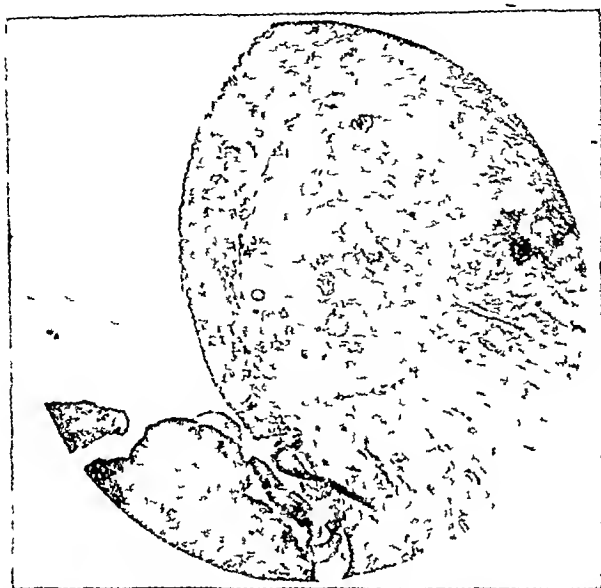


Fig 2—Tuberculosis of the synovial membrane. In this patient the resistance was more active. Encapsulation of the tubercles is evident and no necrosis. Elaborate classifications have been built up on the difference in the proportions of invasion and repair.



Fig 3—Old cheesy, encapsulated tuberculous focus dug out of the fibrous adhesions in a resected ankle. The joint had been treated with apparatus years before and had been considered cured.

be demonstrated. In them the formation of scar tissue may prevail. Adhesions form in the synovial membrane.

The cartilage becomes thinned, and the synovial membrane substitutes it at its circumference, and spreads out over its surface, giving the appearance

of a perichondrium. With the interference with the function, the cartilage itself becomes fibrous. In severe cases the cartilage may be bound tightly to the synovial membrane by adhesions and to a lesser extent to the opposing cartilage. The joint cavity then is replaced by a mass of scar tissue, fibrous ankylosis. In some infections this fibrous ankylosis may become bony. In a supposedly cured tuberculous joint collections of cheesy material may be locked up for years in the fibrous adhesions ready at any time when injury or unwise operative interference sets them free to light up the disease afresh.



Fig 4.—Tuberculosis of the marrow immediately under the cartilage. Fingers of tuberculous granulations are beginning to push up through the cartilage. The cartilage is degenerating and has become fibrous at its surface.

(Fig 3) The muscles proximal and distal to the joint atrophy and various circulatory changes may be added. On the other hand, as the result of appropriate treatment, or spontaneously the disease may die out and perfect function may return, even after what appears to be extensive change. This last occurs rarely if ever in a tuberculous joint.

When the disease starts in the marrow, it gains the under surface of the cartilage, the granulations absorbing or killing the bone trabeculae as they spread. They push up fingers through the cartilage (Fig 4), and spread along its under surface (Fig 5), interfering with its nutrition and perhaps

killing it, gaining the joint either through it, or at its circumference. As soon as the synovial membrane is involved, it reacts as described above.

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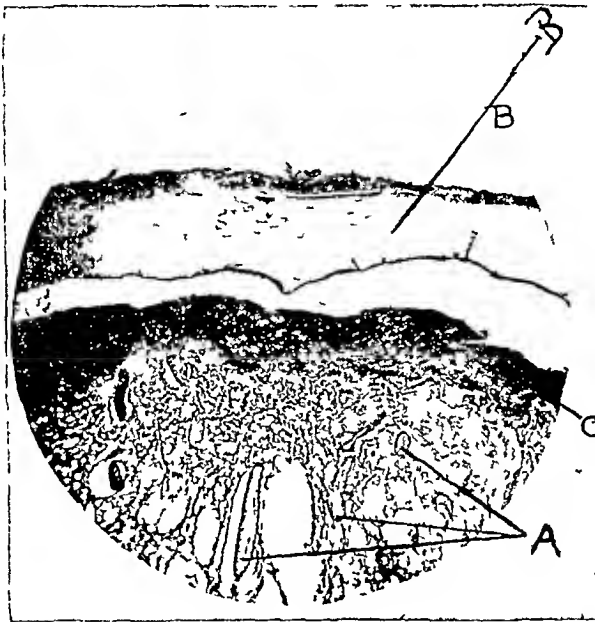


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While no age is exempt arthritides of this type are distinctly diseases of the earlier periods of life, in contradistinction to second type arthritis, which never occurs before the third decade rarely then and increases in frequency as age advances. The prevalence of lymphoid marrow in the bones of young persons explains the occurrence of certain of them notably of tuberculosis and of syphilis. Necropsies in cases of typhoid have shown the constant presence of typhoid bacilli.² Acute suppurative hematogenous osteomyelitis is simply an inflammation of lymphoid tissue locked up in bone, and is therefore more frequent in young persons.

The x ray characteristics of the type are swelling of the soft parts thinning and irregularity of the joint space and rarefaction of the bone. By the extent and distribution of this rarefaction the skilled radiographer is wont to make a shrewd guess as to the identity of the causal infection. In tuberculosis the rarefaction in the earlier stages may appear as a small area at the side of the film, just below the cartilage or perhaps as a narrow streak beneath a portion of it. In the later stages the whole end of the bones may appear irregularly destroyed. This irregularity is a feature of tuberculosis. Bone production does not occur in the region of the joint in this type of arthritis. The light areas in the films of slow old cases of tuberculosis are due to deposits of calcium salts not to bone. In all the slides of tuberculous bones I have examined I remember to have seen an indication of new bone formation in only one, and that was too small to have cast a shadow. The radiographer draws his conclusions from the shadow cast by the container of a sick tenant.

DIFFERENTIAL DIAGNOSIS

All these cases look alike. We strive first of all to place the case into the type to which it belongs and then to find out its identity. This is never possible without the demonstration of the causal organism. We rely for a working hypothesis upon the history and upon a general examination of the patient. Each member of the class has peculiarities which enable us to recognize it in a large proportion of cases. When after a long clinical experience, we think we can do more than this, we err. Ignorance of this fact is responsible for many extravagant claims of cure. Following are a few general rules.

Tuberculosis is uniaxial and shows a preference for the larger joints. Muscular spasm and muscular atrophy are early and marked. The disease is irregularly progressive. The patient may be better this week than last but next month he will be worse. Family history is important, not so much for the tuberculous diathesis on which we formerly laid such stress, as for the infection spread by a careless relative or for the infection of several members of the family by the domicile or by the dairy herd. The tuberculous "habitus" is also unreliable. Many patients with tuberculous joints are otherwise strong and well. On the other hand the presence of an active tuberculous lesion elsewhere in the body naturally influences us in our diagnosis.

Chronic Gonococcic Arthritis—This is a misnomer. A gonococcic arthritis is probably always acute. The gonococcus does its work quickly, and dies out. What is known as chronic gonorrheal arthritis is caused by some other organism, probably by a streptococcus from a secondary infection in the deep urethra, following a gonorrhea. It is almost always multiple. The knees and feet are often involved, not only in their joints, but also in the bone directly under the tubercle of the calcaneus. In this case the pain may be great. The patient walks as if he were treading on eggs. Some cases of intractable old spinal arthritis are almost undoubtedly due to a lesion in the deep urethra.

Arthritis from *infection in the tonsils* frequently affects the joints of the hands and feet, and is also wont to be multiple. When the fingers are affected, the metacarpophalangeal and the proximal interphalangeal joints are the ones involved, in contradistinction to the arthritis of the second type, which affects the terminal interphalangeals. The knees suffer often.

Syphilis of the joints may imitate almost any form of arthritis of this type. It may be painful, contrary to the general idea, or painless, as in the knee synovitis of the late stages. It may involve the bone alone, or the synovial membrane. If the bone be involved, the synovial membrane usually escapes, in distinction to the other infections. In these, when the marrow in the bone end is diseased, the synovial membrane regularly participates. A positive Wassermann test is an indication, nothing more. A negative test means little. Hence the importance of the therapeutic test in the diagnosis.

The arthritis of *coccidioidal granuloma* imitates exactly that of tuberculosis. I know of no way to distinguish them except by the demonstration of the causal organism.

Typhoid arthritis and *pneumococcic* arthritis are rare. They can rarely be missed, if one keep in mind the possibility of an arthritis from the causal organisms during pneumonia or typhoid, or immediately following one of them.

From arthritis of *the second great type* these arthritides are distinguished by their more active inflammatory nature. Usually they are much more painful. The ends of the articulating bones are not enlarged. The deformity is wont to be greater. The limitation of motion is caused by muscular spasm, and by adhesions in the joint, whereas in the second type it is caused by a mechanical obstruction from the change in the shape of the bone ends. In second type arthritis of the hip, however, the pain and muscular spasm may be great, but here the attitude of the limb, the age, and the history of the case may help us out. In arthritis of the first type a fairly constant relation exists between the symptomatology and the anatomic change, but not in arthritis of the second type. We usually rely on the x-rays for a final opinion. These are almost always conclusive, if we know the pathology of the two diseases. Bony spurring and hiping is present in arthritis of the second type, absent in that of the first. This cannot be recognized in the fingers, and may be imitated in the spine and foot by a peculiar moulding of the

joint margins. Occasionally the detection of the characteristic cavities in the bone ends later to be described will help us to recognize the identity of a second type arthritis, when otherwise we should go astray.

TREATMENT OF TYPE I ARTHRITIS

We cannot go into the details of treatment here. They have been set forth fully in my book.² A few main principles will suffice.

All arthritides of this type we assume are due to a focus of infection somewhere else in the body. They immediately fall into two classes. Those which, like tuberculosis, find a suitable habitat in the joint tissues, and are capable of indefinite existence independent of the original focus, and those which, like syphilis, and the infections from the tonsil and from the deep urethra, recover promptly when the distant focus is cured.

Tuberculosis—All the pathology and symptomatology show us that nature is expending every effort in one direction, to deprive the joint of function. We follow her and take this as our first great rule of treatment. A study of the pathology teaches us further that nature unaided is unable to deprive the joint completely of function; that is to accomplish a bony ankylosis, unless a secondary pus infection be added. We should expect then that unless by our treatment we completely destroyed the joint, we could not hope to cure the disease. Clinical experience, by its confirmation of laboratory study, teaches us that this is a fact. When I stated this as an axiom twenty years ago it was ridiculed or ignored. It has slowly made its way, and is now widely accepted though with little credit to the author of the theory. While I admire the enthusiasm of those who believe that they can cure tuberculous joints by sunlight, sea air, injections or apparatus, I cannot share their faith. It is just as easy to replace the cartilage on the end of a bone as it is to make hair grow again on a bald head. If the disease be located exclusively in the synovial membrane, cure under conservative treatment is perhaps possible.

Uncomplicated joint tuberculosis is a disease exclusively of lymphoid marrow and synovial membrane, with little or no effect on the general health. When a secondary infection is added, it becomes a widespread and very dangerous disease, with marked constitutional involvement. From this we deduce our second rule—Avoid secondary infection. A tuberculous joint in communication with the outside almost invariably becomes infected. Therefore we do our utmost to prevent this communication. We never open drain, or scrape out tuberculous joints, for we know well that in attempting to provide for the exit of tuberculous material we shall really provide for the entrance of pus germs.

Syphilis—The treatment of syphilitic arthritis is constitutional, with the usual antisyphilitic drugs. A syphilitic joint should not be invaded by the knife.

Arthritis caused and kept alive by a focus in the tonsil or in the deep urethra in the male. Removal of the causal infection cures these cases. My experience with the removal of any other foci than these has been very dis-

appointing I believe that the teeth have nothing whatever to do with this form of arthritis. Infection at their roots is a contributing factor in an entirely different form of arthritis.

Typhoid—The chronic form of typhoid arthritis usually occurs in the spine. With rest and patience, the patient always recovers.

Coccidioidal Granuloma—Little in the way of treatment can be done for this. The prognosis is very bad.

Some desperate cases of the multiple form of arthritis, in patients with intestinal infestation by protozoa. I have seen improve markedly or even recover completely under treatment directed against the protozoa. To cure a patient with this disease is a triumph, but I am not attempting to claim that I have made a great discovery. It is a good thing to remember as a last resort.

Certain other cases of the progressive form will defy all our efforts. We are unable to find out their cause. In these circumstances we are compelled to fall back upon pure empiricism. We sometimes try vaccines of various sorts, and I have seen some good results from them, but as a rule we are disappointed. Others are more enthusiastic. Much the same may be said of treatment by physical therapy.

Treatment by Diet—Any one who has had much experience with obscure cases of chronic arthritis has observed that the symptoms are aggravated by anything which disturbs the digestion, and that his patients do much better on a diet which agrees with them. Upon this observation many investigators have attempted to found a scientific treatment of the disease. The pendulum swings back and forth. A few years ago, the talk was of purin metabolism, and the uric acid diathesis. Meat was taboo. The present vogue of carbohydrate intolerance reminds of the Salisbury diet of beefsteak and hot water of fifty years ago. It is probably more rational than the other theory, for lean, slender persons, the carnivorous type of human, are generally those who have this form of chronic arthritis.

One of the most popular methods of treating patients with chronic arthritis is to send them to some one else. The various spas and health resorts form a convenient resort for them. The management regulates the diet and mode of life, prescribes plenty of water to drink, regulates the bowels, and applies heat in some form, always grateful to those with painful joints. Under these conditions the patient usually improves, but the improvement is temporary.

THE SECOND GREAT TYPE OF CHRONIC ARTHRITIS

This is a separate and distinct entity, whose gross anatomic change is a piling up of bone and cartilage at the circumference of the joint cartilage, along the line of the attachment of the capsule, so-called spurting and lipping (Fig 6). By these masses of new bone and cartilage the disease is recognized clinically, and from them it usually derives its names, many in number. Its occurrence is widespread, and it is of ancient origin. Skeletons dug up from ancient Egypt are said to exhibit its distinguishing marks. It

affects persons of middle and advanced age, and rarely occurs before the fourth decade of life. I have never seen a case before the third decade, and in an experience with hundreds of cases, only two or three in that

ETIOLOGY

The two most popular causes ascribed to this disease are (1) trauma and (2) infection. Before discussing the arguments for these a word may be said concerning the functions of bone.

Bones have two separate and distinct functions: a mechanical function, as the framework of the body, and an entirely different function, that of container of a very complex and temperamental tissue, the bone marrow. In its first function bone can be injured in only one way. It can be fractured. There can be no such thing as a 'slight' injury of bone. A trauma fractures the bone or leaves it unscathed. As the container of the marrow, bone may

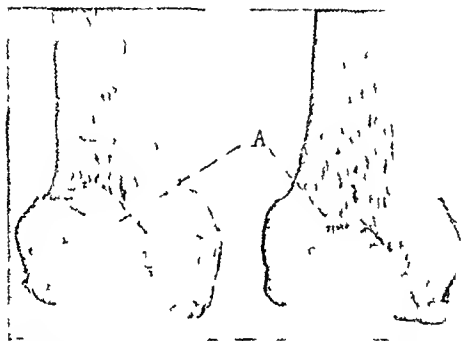


Fig. 6.—The ridges of new bone (1) piled up at the circumference of the joint capsule in second type arthritis.

suffer absorption, death, or increase in size or in density. It is well known that infections in bone do not follow closed fractures. On the other hand, slight injuries of bones are often said to predispose to infections. As there can be no slight injuries, this is impossible. If bone, however, suffers absorption and death from infection in its contained marrow, its weakened structure does in fact predispose to fracture.

If the capsule of a joint be torn, an exudate is poured out in the joint cavity, and the usual inflammatory processes of repair ensue. Infection of the joint tissues is no more to be expected than is, in like circumstances, infection of any other tissues remote from the surface of the body. Trauma does not predispose to joint infections.

Trauma—The chief reasons for believing that trauma does not cause arthritis of the second type are (1) It is not possible to explain the anatomic changes observed in the joint by any trauma of which we can conceive. There must be a relation between cause and effect, and this is not present

here, (2) the joints of children and of vigorous young adults are much more liable to injury than are those of elderly persons, and yet they are invulnerable to the disease, (3) experiment fails to establish any relation Dr Cowan and I injured the joints of many rabbits in various ways, and in no instance did we succeed in causing the disease⁴

Bacterial Infection—Various investigators claim to have recovered bacterial organisms from these joints Others deny that they are ever present I have never succeeded in recovering any organisms except possibly staphylococci, undoubtedly contamination Dr Dickson, head of the bacteriologic laboratory, conducted the investigation for me

The chief argument against the infection theory of the causation of this disease is one which unfortunately will be appreciated only by one familiar with the reactions of the joint tissues to disease The changes observed in the joint tissues are absolutely and completely different from those caused by bacteria Without wishing to appear frivolous, I view the arguments of those supporting the infectious theory with the same scepticism with which I should view the claim of one that a woodchuck burrow was caused by birds because he had found a brood in the hole The changes produced by bacteria are all about the same, and these are entirely different

I believe that this type of chronic arthritis is caused by protozoa, probably by one or by several of the so-called harmless varieties, which gain access to the circulation almost invariably through the open bone at the root of dead teeth My reasons for this belief have been set forth in numerous publications⁵ They may be summed up here

Investigation has convinced me that the first and fundamental change in this type of arthritis, as will appear below, is aseptic necrosis in the marrow near the articulation All the other changes are caused by this This necrosis must be caused by some living organism It is not caused by bacteria I know of no microorganism other than a protozoon capable of producing this aseptic necrosis, and this is just the sort of lesion which a protozoon of low virulence would cause The stools of a heavy proportion of patients with this disease contain protozoa The patients almost always show evidence of past or present infection at the roots of their teeth Only about one per cent are without it This explains the increasing frequency of the disease with advancing years This theory lacks the keystone of its arch, the demonstration of the organism in the marrow I realize that no amount of therapeutic results will make up for the lack of this

MORBID ANATOMY OF TYPE II ARTHRITIS

The gross pathologic changes have been known for a long time, the finer ones were worked out by Nichols and Richardson⁶ The ridges of new cartilage and bone (Fig 6), piled up at the circumference of the articulating bone surfaces, are characteristic, and are responsible for most of the names bestowed on the disease The joint cartilage becomes fibrous and calcified (Fig 7) It degenerates, and then disappears over larger and smaller areas, leaving the underlying bone bare (Fig 8) This bone becomes thickened

and dense, "eburnated" ivory like, grooved in the line of joint motion. This layer of bone prevents the communication of the marrow spaces below with the joint cavity so common in first type inflammations. We miss here also the formation of scar tissue, and the adhesions of the capsule with the bone ends, and of the bone ends with each other.

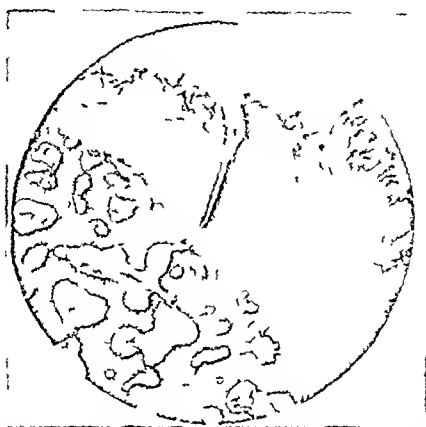


Fig —Degeneration fibrillation and calcification of the articular cartilage



Fig 2 —Thickening of the bone immediately under the joint cartilage. The cartilage has disappeared leaving the bone bare. Compare this photomicrograph with those of tuberculous joints. The two processes are absolutely different.

The grinding and grating of the bare bone ends has led to a rather popular idea that these joints are dry. On the contrary they often contain much free fluid. As there is no direct connection between the bone ends the restriction of motion is due to mechanical factors through the change in their shape. In the spine however, masses of new bone are formed under the common ligaments, like syrup poured from a jug and then solidified. These

fuse two or several vertebrae. The characteristic changes also take place in the lateral articulations, and this should be emphasized, for they are of importance in the symptomatology. A spondylolisthesis is seen occasionally. In the shoulder the rubbing of the long head of the biceps against the rough humeral head may fray it out and may finally divide it. In the fingers the terminal interphalangeal joints become knobby and swollen, with lateral and flexion deformities—Heberden's nodes—and are often mistaken for gout. The fingers are often affected, the toes, except the first, more rarely.

The changes in the synovial membrane are those one would expect from a long continued series of mechanical insults. They are quite different from the so called lymphoid proliferation of the first type. The membrane becomes greatly thickened, and this thickening is due to a production of loose

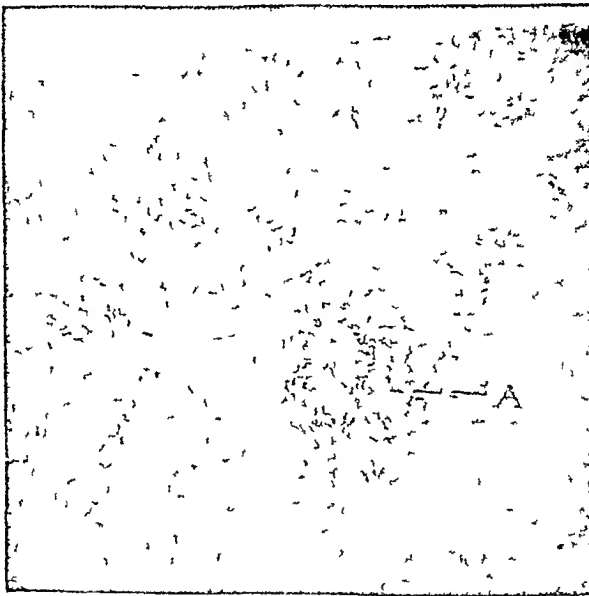


Fig 9—Synovial membrane from a case of second type arthritis. Note the thickening due to fibrous tissue and fat. One area of cellular infiltration at A.

meshed fibrous tissue and fat (Fig 9). A small cellular production takes place at the surface, giving it, to a superficial microscopic examination, the appearance of columnar epithelium. The synovial membrane loses its smooth, glistening surface, and becomes a mass of greatly enlarged villi, branching and dividing like moss on a rock. To these villi, and to the thickened capsule, the swelling of the joint is partly due. The rest may be caused by the enlargement of the bone ends. Cartilage and bone may form in the villi, and such villi, becoming twisted off, may form joint mice (Fig 10).

Most of these changes have been known for some time. The peculiar thing was that we could not make out why they took place. Given the original tubercle in the bone marrow near the joint, everything thereafter is perfectly understandable, but here we seemed to be viewing a collection of random morbid changes. We could not understand what set them in motion.

Cysts have been noted in the marrow near the bone ends by several observers. Nichols and Richardson attributed them to injury, but it is hard to understand how injury could possibly cause them. In point of fact these cysts hold the key to the problem.

ASEPTIC NECROSIS THE PRIME PATHOLOGIC CHANGE

If the end of a bone removed from a patient with this disease be sawed into slices, areas of aseptic necrosis of bone and marrow will be found (Figs 11 and 12). They are entirely different from the necrotic areas found in tuberculosis for instance. They may be large or small (Fig 13). Sometimes practically the whole bone end may be dead. Possibly a sequestrum may be broken off from it as in one of my specimens. In this specimen, interpreted by Dr. William Ophuls as an early form of the disease, cysts were not present.



Fig 10—Second type arthritis. Resected head of femur. Note characteristic flaring at L. Most of the bone is dead. Sequestrum at S.

ent? Usually cysts more or less discrete are seen, perhaps surrounded by thickened bone, often with fibrous septa. Sometimes the fibrous tissue predominates, with larger and smaller cysts in it. Different sections from the same area may show varying proportions of fibrous tissue and cystic degeneration. The sequence is probably this: necrosis, vascularization, fibrous tissue, cystic degeneration.

In direct contrast to her methods in first type inflammations nature now attempts, and usually succeeds in walling off the disease from the joint. She builds a bony wall, more or less complete, around the areas of necrosis and thickens the bony buttress beneath the joint cartilage. The cartilage degenerates and wears away. The new bone at the circumference of the cartilage is either part of this same process, or perhaps is caused by the strain on the capsule at its line of attachment, due to the changed shape of the bone end. All the changes in the synovial membrane may be explained by continuous trauma, repeated small sprains.

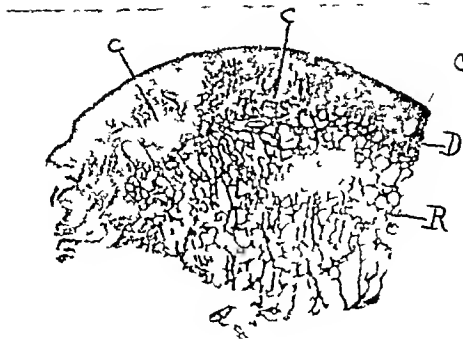


Fig 11—Cavities in bone (C) two of them filled with fibrous tissue Eburnated bone at D Marrow at R

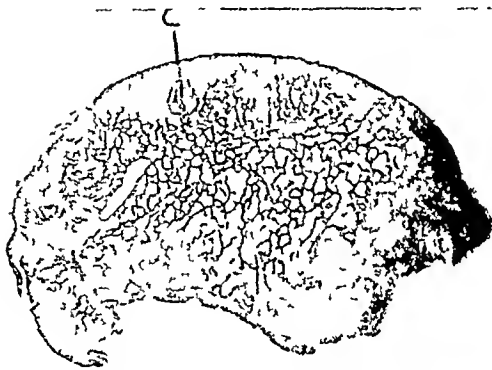


Fig 12—Same specimen as Fig 11 Section cut a short distance away

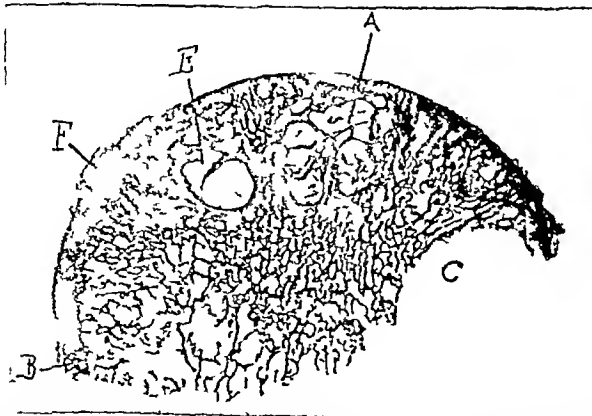


Fig 13—Cavities in bone Aseptic necrosis in bone C Large cavity cut through in resecting the head of the femur F Area of fibrous tissue E fairly large cyst A large cyst with many fibrous septa. B, New bone forming in joint cartilage

SYMPTOMATOLOGY OF TYPE II ARTHRITIS

This disease has certain peculiarities. It is distinctly a disease of middle and later life and affects those with dead teeth. The inflammation is of a lower grade than is the arthritis of the first type. There is absolutely no correspondence between the amount of anatomic change and the symptoms and physical signs. Extensive bone changes for instance may be discovered in the spine by films taken for disease of the viscera. On the other hand with slight anatomic changes the pain and disability may be great and the physical signs may be marked. The knowledge of this absence of correspondence between anatomic damage and physical signs is important in estimation of disability after industrial accidents. In the past when x ray films taken shortly after an accident showed the characteristic hipping of the disease the disease was assumed to have resulted from the accident, and compensation was assessed accordingly.

To give in detail all the symptoms and physical signs of this disease is unnecessary. When however the disease occurs in the spine it possesses certain peculiarities. A lack of knowledge of these has given rise in the past to great confusion. Even today it is responsible for many erroneous diagnoses.

CHRONIC ARTHRITIS OF THE SPINE

In chronic spinal arthritis of this type the symptoms and physical signs may be severe, mild or absent. With marked anatomic change as revealed by the x rays the spine may show a normal contour and perfect flexibility. Again, with minimal changes, the pain and stiffness may be great. The pain may be felt in the back 'lumbago'. More commonly it radiates out along the spinal nerves. It is a true referred pain like that which a patient feels in his foot after his leg has been amputated. It is not caused by any pressure upon spinal nerves in the spinal foramina. Painful impulses coming in from the spinal articulations are referred to nerve tracts which enter the spinal cord at the same level and over which sensations from the periphery usually come.

In these cases of spinal arthritis when the pain is felt in the back, it is usually diagnosed myositis, fibrositis or fasciitis. If the medical man remove a piece of tissue for examination, the 'round cell infiltration' he finds may confirm him in his error. If the pain be referred out along the upper extremity in cases of cervical arthritis it is usually diagnosed and treated as neuritis. Neuritis has been a very popular diagnosis in recent years. It has even partly displaced the diagnosis of rheumatism among the laity. Now, true neuritis is a definite anatomic thing which lasts a long time. The motor fibers being more labile suffer first and principally, while in these neuritides the pain comes and goes, and motor symptoms are conspicuous by their absence.

When the pain was referred to the lower extremity it was called sciatica. It was treated with antirheumatic remedies by injection, or by external applications. Perhaps the "adhesions" which were supposed to bind

the nerve to the surrounding tissues were broken up by stretching, either dry stretching or bloody. If the sciatica was due to the little known lesion of pubic subluxation (the so-called sacroiliac slip), these procedures were probably successful, though the results of bloody stretching of the sciatic nerve can be much more simply obtained by superextension of the thigh on the pelvis.

The latest diagnosis of these peripheral pains is radiculitis. While I believe that the diagnosis is purely fanciful, I know of no way at present to prove that an inflammation of the posterior roots does not exist. The condition does not differ essentially from that existing in tuberculosis of the spine, yet we do not diagnose a radiculitis with the so-called root pains there. We recognize the fact that the inflammatory process in the vertebrae can explain the referred pains, and that they will disappear when motion ceases at the diseased area. I suspect that radiculitis will follow neuritis and myositis, etc., into oblivion, and that then we shall hear of posterior poliomyelitis.

TREATMENT OF TYPE II ARTHRITIS

Most patients with this form of arthritis are relieved by the application of heat in some form. The tendency of persons with senile rheumatism to hug the fire is well known. Errors in diet aggravate their symptoms. No two persons are the same. Certain of them are meat eaters, and others thrive on carbohydrates. Some things are proverbially bad for "rheumatics," notably shell fish, strawberries and tomatoes. One dietary fad will help one patient, and harm another. Tea, coffee, tobacco, and alcohol are poisons which most persons tolerate fairly well. If any patient have an idiosyncrasy against any of them, however, his joint symptoms will improve when his medical adviser cuts them off. The various forms of physical therapy are often grateful to the patient. In no circumstances, however, should any vigorous measures be carried out. The disease has already damaged the joint mechanically, and forced exercise, active or passive, injures it further.

The treatment which we carry out at the orthopedic clinic at Stanford, and which is employed more or less in this part of the country, may be inferred from what has been said of the pathology and the etiology. We assume the cause to be some form of protozoon, probably one of the so-called harmless protozoa, domiciled in the gastrointestinal tract, which gains entrance to the circulation through the open bone at the roots of dead teeth. The first steps in the treatment are the extraction of any dead teeth, and the identification of the protozoa if they be present. This last is no easy task, and should not be entrusted to a tyro. No one lacking a thorough training should undertake it. Fortunately at Stanford we have a trained laboratory man, Dr. Harry Wyckoff. Our routine consists of the examination of specimens of five or six stools, done on successive days.

In mild cases, when the stool examination is negative, we usually wait to see the results of the extraction of the teeth. Sometimes the symptoms subside, and no treatment is necessary, if not, we give a course of emetin.

The routine treatment for *Giardia* infestation is three injections of neoarsphenamine, at intervals of two days. With other protozoa we give the full course of neoarsphenamine and emetin.

Emetin is a powerful and a dangerous drug. Anyone who employs it should observe certain precautions. We had one or two unfortunate experiences in our earlier treatments. Following are a few rules which I should emphasize.

1 No patient with vascular disease should have emetin. High blood pressure is a contraindication.

2 While taking emetin the patient should be seen every day. His blood pressure and his pulse should be noted. With an irregular pulse the patient stops his treatment. A falling blood pressure and a rising pulse indicate caution. With a pulse rate of 90 the patient should be in bed. If it reach 100, the treatment should cease at least temporarily.

3 The diet should be regulated carefully. Only the most digestible food is permitted. Raw fruits are interdicted and no cathartics are allowed.

4 At the first sign of diarrhea the treatment stops.

5 At the first sign of muscular weakness the treatment stops.

6 Vomiting is a contraindication but this can usually be avoided with care.

7 The usual precautions should be observed of course if neoarsphenamine be employed.

Our results with the use of this treatment have been good, better than those I have observed with any other treatment of this form of arthritis. There are certain limits to the efficacy of any form of treatment. In the first type of arthritis if the cartilage has not gone, a retrogression of the anatomic changes may occur, and a return of good or even of perfect function. In this second type the anatomic changes are permanent. The most we can do is to bring them to a standstill. In other words a patient who once has this disease in his joint never has a perfect joint again.

CONCLUSIONS

1 An arthritis is a synovitis, and a synovitis is an arthritis. The marrow in the vicinity may or may not be involved.

2 An inflammation of the synovial membrane like that of any other organ, may be caused by any one of a number of things.

3 Many arthritides are caused by infections with bacterial organisms carried to the synovial membrane from some other place in the body, the so called foci.

4 In certain cases the joint tissues are capable of conquering the infection. In these, when the supply of bacteria is shut off by the removal or cure of the infection, the arthritis recovers. Syphilis, gonococcal infection, and infections from the deep urethra and from the tonsil are examples of this.

5 In certain cases the infection, once domiciled in the joint, is capable of independent existence for an indefinite time. Tuberculosis and coccidioidal granuloma are examples of this, probably many obscure cases also.

6 All these infectious arthritides have a common symptomatology and a common pathology. Other cases, with a similar pathology and symptomatology are probably also infectious in their origin.

7 The teeth have nothing to do with this type of arthritis. From clinical observation I believe that the sinuses, the anti, the ears, the appendix, the gall bladder etc. are guiltless of any causal rôle. Depending as it does upon pure clinical observation, this finding is possibly wrong.

8 The second great type of arthritis is sharply differentiated from the first, symptomatologically, radiographically, and pathologically.

9 The cause of this second form is not known. The disease cannot be caused by bacteria, or by trauma. Laboratory examination of material strongly indicates that it is caused by some living organism. I believe that this organism is probably a protozoon.

10 Clinical investigation indicates that this protozoon may be one usually considered harmless domiciled in the intestinal canal which usually gains entrance to the circulation through the open bone at the roots of dead teeth.

11 The best form of treatment of this arthritis is the extraction of the dead teeth, followed, if necessary, by the administration of parasitocides.

12 The blind and ruthless extirpation of so-called foci for the relief of pain, without an exact knowledge of the underlying pathology, and without a knowledge of the possible relation which these foci bear to the arthritis, is here, as with inflammations in the other organs of the body, unnecessary, unscientific, and often harmful.

13 Many problems remain in chronic arthritis to be solved. The solution will not be advanced by the use of vague terms which disguise our meaning or hide the fact that we have no meaning to disguise.

14 Most cases of so-called neuritis, fibrositis, myositis and fasciitis are really cases of spinal arthritis. The same may be said of the new disease, radiculitis.

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STANFORD UNIVERSITY, SCHOOL OF MEDICINE

AN ANALYSIS OF THE RESULTS OF TREATMENT OF CASES OF TYPE II ARTHRITIS IN THE STANFORD CLINIC*

By PAUL A. DYELBA, A.B., SAN FRANCISCO

IN INTRODUCTION, it must be said that no attempt has been made to study the etiology or the pathology of this lesion of the joints which has long defeated medical endeavor. The principal interest of the work was in studying the results of emetine therapy on the condition. Statistics of other types of therapy introduced are for the purpose of serving as controls on the results of emetine.

By Type II Arthritis is understood that form of arthritis which most often appears insidiously in the later decades and leads to the lipping and spurring around the joints which has given it the commonly used name of hypertrophic arthritis.

The age range of the 149 cases studied closely parallels that given in standard works for the condition. There were 2 twenty eight year old patients these being the youngest in the series. There were 23 patients in the fourth decade, 45 in the fifth, 53 in the sixth and 27 in the seventh decade. There were 78 per cent of the entire number of cases between the ages of forty and sixty five. There were 57 per cent of the cases females, 43 per cent males.

The pathologic findings in the joints have led to the assumption that protozoa situated in the bone in the immediate vicinity of the joints are the causative agents of this type of arthritis. It is on this assumption that the emetine, neocarsphenamine treatment is carried out.

When the diagnosis of Type II Arthritis is made the patient is sent on for stool examinations on five successive days, and for a thorough dental examination. Any dead teeth are extracted. In the event of parasites being found in the stool, neocarsphenamine is combined with the emetine. Otherwise emetine is given alone. After the stool examination the patient receives an examination of the heart any disturbance in cardiac function being taken as a contra indication to emetine treatment. It is of interest to note that of our 149 cases 7, or 4.5 per cent, were found to be unsuited to emetine treatment.

The regular course of emetine consists of twelve injections of emetine hydrochloride, a grain being given every day except for the initial dose which is a half grain. The blood pressure and pulse are recorded with each injection which latter may be either intramuscular or intravenous. A marked drop in blood pressure or rise in the pulse rate is taken as an indication to arrest the treatments until the cardiac function is more nearly normal. Diarrhea or muscular weakness are also taken as indications for discontinuance of the treatment.

Submitted to the Faculty of the School of Medicine of Stanford University in partial fulfillment of the requirements for the degree of Doctor of Medicine

Following the emetine injections a course of 15 grams of emetine bismuth iodide is given orally, a gram or two daily, depending on the patient's ability to tolerate the drug. Sixteen grams was the maximum received by any patient in this series. In case the stools are found to contain parasites, neosalvarsan, 0.45, 0.6, and 0.9 grams is interspersed with the emetine injections.

Of 134 patients whose stools were examined, 37, or 27 per cent, were positive for parasites and 97 or 73 per cent were negative. It should be stated that while the majority of the negative cases received five examinations, all cases that received any stool examination, and in which no parasites were found are considered negative. On the other hand, the positives, while being chiefly *Endameba coli* include five other parasites as well.

As it is thought that infected teeth may play some part in the disease, the results of the dental examinations are of some interest. In this series of cases, 68 patients showed one or more abscessed or dead teeth, or root remains in the jaw. In 49 cases the dental examination was negative and 36 cases received no examination. Many patients were edentulous.

The patients considered in this paper received the following treatment: 63 received emetine, 41 received no treatment at all, the reason in the majority of cases, being manition on the part of the patient. Seventeen had infected teeth removed, 10 had foreign protein therapy in the form of gonococcus vaccine, 5 cases received braces, 5 physiotherapy alone, and 5 neosphenamine alone. Two cases were treated with aspirin and one with emchophen.

Of the 63 patients treated with emetine, 5 or 8 per cent, received complete relief of symptoms, 12 or 19 per cent, received considerable relief, 12, or 19 per cent, received slight relief, and 14 or 22 per cent, received no relief. Nothing could be found of the results of treatment in the remaining 32 per cent of cases. These figures show that in 67 per cent of the cases where the results are known emetine therapy gave some relief of symptoms, and 12 per cent of this group obtained complete relief.

Of the 41 patients who received no treatment, the results are unknown in 38. Of the remaining 3 patients, 2 remained the same, one became worse.

Of 9 of the 17 patients who had teeth extracted and no other treatment, nothing is known. Of the remaining 8 patients, one claims permanent complete relief, one considerable relief, 2 slight relief and 4 no relief.

Of the 10 cases of foreign protein therapy (vaccines), 3 patients obtained considerable relief, 2 obtained slight relief, 2 no relief, and the remaining 3 were not determinable.

Of the 5 patients treated with braces, 2 obtained considerable relief, one no relief, and the results in the remaining 2 cases are not known.

Of the 5 patients treated with neosphenamine alone, one patient received considerable relief of symptoms, one patient slight relief, one patient no relief, and the results in the remaining 2 cases are unknown.

Nothing is known of 3 of the 5 patients who received physiotherapy. The remaining 2 patients received no permanent benefit.

Of the two patients treated with aspirin, one received complete, permanent benefit, the other patient receiving only temporary relief. The results in the case of the patient who received emchophen are not known.

It is usually conceded in papers on the treatment of chronic arthritis that the success of therapy is in inverse proportion to the duration of the process before treatment is started. With this fact in mind the results of emetine treatment have been arranged in Table I.

TABLE I
DURATION OF THE PROCESS

	3 MO. AND LESS	6 MO. AND LESS	1 YR. AND LESS	2 YR. AND LESS	5 YR. AND LESS	5 YR. AND OVER
	%	%	%	%	%	%
Complete Relief	2 20	0 0	0 0	1 20	1 17	1 14
Considerable Relief	3 30	75	0 0	0 0	2 25	4 58
Slight Relief	2 20	1 25	4 45	2 40	2 25	1 14
No Relief	3 30	0 0	5 55	2 40	3 38	1 14

It can readily be seen that this table fails to show, even roughly, an inverse ratio between the number of successful cures and the duration of the process. That is, in this series of cases the prognosis for the patient whose disease has been present for years is as good as that for the patient whose arthritis has been present three months or less. A larger series of cases might show different results.

As the full course of emetine consisting of 12 injections and 18 grains orally, is rather prolonged, it is of value to determine the results if any of an incomplete course. Table II compares the results of therapy of varying degrees of completeness.

TABLE II

BELIEF	EMET GR. 5	EMET GR. 8	EMET GR. 12	EM GR. 12 EM. B. I.	EM GR. 12 EM. B. I. NEO	EM GR. 6 EM. B. I.	EM GR. 6 EM. NEO
Complete	0	0	2	3	0	0	0
Considerable	2	0	4	2	2	0	2
Slight	4	0	1	5	1	0	1
None	1	1	4	3	4	1	0

This table indicates that the regular 12 grain course of emetine gives better results than do shorter courses. Considering the three groups which received 12 grains or more of emetine the results are: complete relief 5, or 17 per cent; considerable relief, 8, or 27 per cent; slight relief 7, or 23 per cent; and no relief in 10, or 33 per cent.

It is, of course, of the greatest importance to know the permanence of our successful treatments, so we have made note below of the time elapsed since the completion of treatment in those cases which received complete or considerable relief.

CASES WHICH RECEIVED COMPLETE RELIEF

Case 45	5 years	Well at the present time
Case 92	3 years	Well at the present time
Case 130	3 years	Well at the present time
Case 143	1 year	Well at the present time
Case 40	2½ years	of complete relief. Pain has been gradually returning for the past year.

CASES WHICH RECEIVED CONSIDERABLE RELIEF

Case 62	5 years
Case 102	5 years Pain has been starting in other joints recently
Case 27	2½ years of considerable relief, after which the pains gradually returned
Case 170	2 years
Case 12	1½ years
Case 147	1½ years of considerable relief Pain has been returning recently
Case 77	1 year
Case 84	6 months

Cases 100, 54, and 103 were not seen at this time but were seen by the Orthopedic Department three months after the completion of their treatment, and at that time had obtained considerable relief.

There is one therapeutic result which must be mentioned though it confuses rather than illuminates, the problem. Case 117 entered the Orthopedic Clinic in April, 1923 with a six months' pain in the knee, and hands which had been painful for ten years, and showed typical second type arthritic changes. For her complaint she was given ispinin. She replies to a letter of May 3, 1930, and states that she received a perfect cure in the Orthopedic Department, and that she is entirely well at the present time. The conditions suggest the possibility of a spontaneous remission.

One of the unpleasant features of the emetine treatment is that certain patients are sensitive to emetine, and show reactions, characterized by nausea, muscular weakness, and general depression. An attempt has been made to analyze the reactions in our series of cases. From the description of the patient's symptoms, given in the chart, an arbitrary classification of the reaction as severe, moderate, slight and no reaction has been made.

Case 82 is typical of the severe reactions. Progress notes from his chart are as follows:

2/12/29 Pains in the abdomen and dryness of throat
 2/16/29 Still weak Shows tremor
 2/25/29 Still weak
 2/27/29 Pains in the back Tired
 3/ 5/29 Headache Testicular pain
 3/ 6/29 Legs and back tired
 3/ 9/29 Still feels badly
 3/13/29 Feels poorly Depressed

Moderate reactions are similar in character but less severe and less prolonged. Slight reactions include any feeling of nausea or weakness temporarily affecting the patient, but passing within a day or so.

Of the 62 cases treated with emetine, 9 had severe reactions, 10 moderate reactions, 15 slight reactions, and 28 no reactions. However, as has been noted above, there were 12 cases which received 8 grains or less of emetine. These must be separated, in considering the reactions, as well as in considering the results of treatment, for it is the effect of the full course of treatment with which we are most concerned.

Of the 50 cases which received a complete course of emetine, 7, or 14 per cent received a severe reaction 9 or 18 per cent, a moderate reaction 13, or 26 per cent, a slight reaction, and 21 or 42 per cent, no reaction. There were 58 per cent of these cases, therefore, which had some evidence of emetine intoxication, and 32 per cent showed moderately severe or severe reactions.

It is to be noted that Cases 36 and 149 received severe reactions after 4 and 6 grains of emetine respectively, so individual susceptibility seems to play a considerable part in the developing of reactions.

To determine the influence of age and sex on emetine intoxication Table III has been drawn up.

TABLE III

REACTION	MALE	FEMALE	FOURTH DECADE	FIFTH DECADE	SIXTH DECADE	SEVENTH DECADE	EIGHTH DECADE
Severe	2	7	3	2	3	2	0
Moderate	4	6	1	5	2	2	0
Slight	4	11	3	4	3	"	0
None	18	13	5	9	9	4	1

It would appear from this table that women are somewhat more susceptible to emetine intoxication than are men. It does not appear, however, that age is of particular importance, the younger patients seeming quite as susceptible to the toxic effects of the drug as the older ones. In fact, from Table III the sixth decade, from the point of view of drug reactions would appear to be the most favorable for the use of emetine.

As a fall in blood pressure occurs in almost every person to whom emetine is given in any amount, an attempt has been made to correlate this fall in blood pressure with the toxic symptoms. The estimation of this fall in blood pressure in the individual case is rather rough, due to the variation in the readings during the few days of the onset of the reaction. An effort has been made to average the readings of the blood pressure at the onset of symptoms, and this is subtracted from the patient's pressure on entry to determine the fall in blood pressure. Table IV compares this fall in the four reaction groups, the figures being from 48 cases on whom accurate blood pressure readings were kept.

TABLE IV

REACTION	AVERAGE DROP IN PRESSURE	GREATEST DROP IN PRESSURE	LEAST DROP IN PRESSURE
Severe	15 mm	30 mm	0
Moderate	22 mm	50 mm	0
Slight	18 mm	50 mm	0
None	18 mm	40 mm	0

This table indicates that for this series of cases at least there is no definite correlation between the degree of blood pressure fall and the severity of the toxic symptoms.

CONCLUSIONS

1 The results of treatment of 108 cases of Type II Arthritis by the Orthopedic Clinic of the Stanford Medical School are noted. Sixty three of these cases received emetine.

2 Of 134 cases of Type II Arthritis which had stool examinations, 27 per cent showed positive results

3 Of 117 cases of Type II Arthritis which had dental examinations, 58 per cent showed one or more dead or abscessed teeth

4 Of the 63 cases treated with emetine, 8 per cent received complete relief of symptoms, 19 per cent considerable relief, 19 per cent slight relief, 22 per cent no relief, and the results are not known in the remaining 32 per cent

5 There is no correlation between the duration of the process and the success of treatment in this series of cases

6 The complete 12 gram course of emetine produced more favorable results than did shorter courses of emetine

7 Of 50 cases which received a full course of emetine, 58 per cent showed some toxic symptoms, 32 per cent showed moderately severe or severe reactions

8 Women seem somewhat more susceptible to emetine intoxication than do men. The age seems to affect the susceptibility but little

9 The fall in blood pressure found in almost all persons taking the emetine treatment seems not to be related in degree to other toxic manifestations of the drug

MT ZION HOSPITAL, POST AND SCOTT STREETS

THE IMPORTANCE OF AN INTENSIVE PROGRAM IN THE MANAGEMENT OF THE ARTHRITIC PATIENT

By WILLARD C STONER * M D , CLEVELAND

THE term arthritis is derived from the Greek ' arthron ' meaning joint and signifies inflammation. Arthritis is one of the oldest diseases of which there is record, having been present in animals before the advent of man. There is evidence to indicate that it is a disease from which man has suffered from time immemorial. It was the disease ' par excellence ' of the ancient Egyptians and the emphasis which the Greeks and Romans put on hydrotherapy indicates that it was a common disability. Today it represents a great economic problem and is as Pemberton states ' one of the great scourges of society '. It ranks next in importance to heart disease and tuberculosis as a chronic disabling disease. Lack of knowledge and lack of satisfactory application of the knowledge we possess of the disease makes it less preventable than tuberculosis.

The treatment of chronic arthritis in the past has been very unsatisfactory, a number of factors have contributed to the lack of satisfactory response to any method of treatment. In the first place our knowledge of definite etiologic factors as applies to an individual patient has very often been wanting and the fact that our general knowledge of the disease has been so limited has encouraged an indifferent or no treatment attitude with the belief that we are dealing with a disease that is incurable. Then too the great variation in symptoms of arthritic and rheumatoid states has tended to add to the difficulty of satisfactory treatment. The rheumatoid problem touches more fields of medicine in its various ramifications than any other disease except syphilis.

Before the days of Trudeau the cure of tuberculosis was regarded just as hopeless as the cure of rheumatoid manifestations. The failure of the medical profession to deal satisfactorily with the problem has encouraged the unfortunate sufferer to seek aid from cultists and faddists of so called medical practice. Fortunately in recent time more and more attention is being given the problem, not only in this country but Europe and special clinics are being set up in the larger centers for the rational treatment of the chronic arthritides. Obviously the problem belongs primarily to internal medicine but the orthopedists have contributed materially to the advancement of our knowledge, not only etiologically but therapeutically.

The problem from the standpoint of treatment is a broad one and success is not obtained by any given procedure but by the sum total of procedures which must be modified according to the individual case response. An effort must be made to correct the disturbed physiology and the sequelae that follow the disease must often be recognized as incurable, such as ankylosed joints or long continued muscle atrophy.

Our knowledge of the disease does not warrant a classification that is definite beyond the two forms that are readily recognized, the atrophic and hypertrophic forms and even these two forms often blend. It would not be profitable to offer a detailed discussion on the various classifications of the disease. Treatment of the disease should begin with a general consideration of the patient from the standpoint of constitutional type, nutrition, anemia, coexisting conditions, life and habits.

A specific etiologic factor to consider is foci of infection, emphasis of which is no longer necessary. A word of caution perhaps, is necessary in the too free acceptance of the belief that all cases of chronic arthritis are due to a recognizable or tangible focus of infection. In a series of 300 cases of chronic arthritis treated in the last seven and one-half years tangible evidence of recognizable foci of infection was woefully lacking. But this should not argue against the importance of removal of every possible focus of infection and in the female the pelvis should not be overlooked any more than the genitourinary tract in the male. Our disappointments with the whole gamut of foci of infections has tended to center our attention more and more on the intestinal tract. Careful roentgenologic work has tended to indicate that departures from normal in the colon characterized by mobile cecum, ptosis, angulation, elongation, reduplication, a greater caliber of the colon, and diverticulosis are rather common. It must be admitted that such a colon represents a congenital defect or may be acquired and probably argues for a constitutional background which predisposes to the disease as may be argued that toxic goiter has a constitutional background which predisposes to the occurrence of the disease. It remains to be seen how much dietary in a specific way may enter into the large bowel changes with the consequent intoxication. These abnormalities are more common in women than men and chronic arthritis is much more common in women than men. In our series of 300 cases there were 94 males and 206 females. It has been shown that coloptosis is three times as common in women as men. Fletcher, Filsbaugh and others have reviewed large series of cases to prove that these abnormalities are commonly found in the chronic osteoarthritic and have emphasized the value of diet, massage, and colonic irrigations.

"Colonic irrigations" seems to be the order of the day and like many other procedures, was first popularized by the faddist and then finally accepted as regular, so that a physician recommending so called colonic irrigations may be permitted to retain his membership in the local medical organization. There is much pro and con discussion to be offered for and against the type and effect of intestinal flora which to date is not definitely proved and a detailed discussion would not be practical within the scope of this paper. Suffice it to say that there is not much evidence to indicate that a change of bacterial intestinal flora alters the arthritic state, and if betterment results, may it not be due to curtailment of diet, particularly carbohydrates. Evidence is at hand that tends to prove that encouragement of relief of colon content has a favorable effect on the disease. Even as radical a measure as colectomy, advocated by Lane, has given beneficial results. Clearing the large bowel regularly of its contents has value,

whether this be encouraged by laxative massage or so called colonic irrigations. However the necessity of passing a rubber tube into the cecum has been over emphasized it generally cannot be done and most of all is not necessary to accomplish the results sought. It is a fact that routine irrigations of the large bowel affect favorably the disease course in certain cases and this therefore must be looked upon as a helpful adjunct in treatment. But it readily lends itself to abuse and quackery. It is found occasionally that foul smelling material passes several days after irrigation which indicates accumulation in certain pockets of the large bowel which may remain over an extended period. Use of bacillus acidophilus organisms change intestinal flora but do not operate differently from the putrefactive type of organisms. We are impressed with the favorable effects of buttermilk (natural) on the rheumatic state of individuals who are not distinctly arthritic. Vaccine therapy as having specific value has been disappointing but nonspecific protein therapy has distinct value in the dormant type of case that does not have marked joint activity. Lactogen intramuscularly seems to be the most acceptable form of nonspecific protein therapy. Typhoid vaccine gives drastic reactions and disappointing responses. Acceptable drug therapy of value consists of salicylates, iodides, emchophen or better neomechophen and amidoxyl benzoate (ortho iodoxy benzoic acid). In spite of the many abuses of intravenous therapy iodides and salicylates are more effective when given intravenously. Amidoxyl benzoate is disappointing in certain cases, the reaction is often profound and occasionally dangerous. It is a marked nitroid reaction with profuse lachrymation and suffocation. Certain cases tolerate it well and are very greatly benefited.

Physiotherapy as applied to the use of heat, massage, hydrotherapy, ultra violet ray, diathermy and faradic current are adjuncts in the routine treatment that may be employed with benefit but should not be applied by the incompetent and extremist. Diathermy is contraindicated in the active joint and favorable results are generally wanting under any condition. The proper use of massage is frequently overlooked. If we accept the theory that suboxidation as a result of impaired circulation to a joint furnishes fertile soil for the disease, then every means at our command to improve this faulty physiologic state should be employed. The problem of dietary is not settled. Obviously the obese and well nourished should have a curtailment of calories which should affect particularly the carbohydrates to lessen the metabolic load. Of course dietary should not be restricted in the anemic impaired nutrition case and there is no scientific evidence to indicate that the dietary shall be other than a balanced ration. For the further relief of pain, stiffness and soreness I know of no combination of drugs so valuable as a capsule containing ext. belladonna, codeine sulphate, camphor monohydrate, acetophenetidin and acid acetylsalicylic.

It may be of interest to briefly review 300 cases treated in the last seven and one half years. Classifying this series according to age it was found that the oldest patient was eighty three years and the youngest was eight years. The average age was forty seven years. The average age of the 94 males was forty eight years. The average age of the 206 females was forty seven years. Of

this series, 163 cases were associated with other diseases and 137 cases were unassociated. Classification of the 163 cases associated with other diseases

- 7 Diabetes
- 35 Obese
- 3 Chronic respiratory infection
- 30 Definite cardiovascular changes
- 3 Toxic goiter
- 3 Hypothyroidism
- 4 Achylia gastrica
- 7 Simple colitis
- 7 Urinary tract infection
- 12 Scatica and synovitis
- 3 Syphilis
- 1 Active pulmonary tuberculosis
- 3 Chronic skin disease
- 13 Psychoneurosis
- 17 Foci of infection such as teeth and tonsils (removal of which had little effect on the disease process)
- 6 Pelvic infections
- 1 Chronic rhinitis
- 1 Periostitis
- 2 Duodenal ulcer
- 4 Marked gastric hyperacidity

The onset was abrupt in 49, insidious in 193 and indefinite in 58. The duration of the disease varied from a few months to thirty-five years. The results of treatment showed marked improvement in 31 or 10 $\frac{1}{3}$ per cent, symptoms relieved but not completely cured in 180 or 60 per cent, temporary improvement with recurrence occurred in 28 or 9 $\frac{1}{3}$ per cent. This represented the type of case that did not completely cooperate over an extended period. Questionable improvement occurred in 43 or 14 $\frac{1}{3}$ per cent. Eighteen cases or 6 per cent were not treated. Approximately 80 per cent were either cured or relieved of active symptoms.

Notwithstanding reported favorable results from sympathetic ganglionectomy and ramisection on certain selected cases it remains to be seen whether this method of treatment will have practical value. Experiences in this problem encourage one to persist in individual patient management, to take advantage of all the means at our command, to improve the patient's general condition, improve his disturbed physiology and thereby lessen the symptoms of the disease and in many cases obtain results that are gratifying. We must make greater effort to recognize the potential rheumatoid subject and by careful study and management prevent the development of serious trouble. If this problem is to be properly handled hospitals must develop special clinics more and more, where a definite plan of treatment will be carried on over an extended period.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE M.D., ABSTRACT EDITOR

TISSUE A Rapid Golgi Method Bubenaite J Ztschr wissenschaft Mikr 46 359, 1929

The author proposes a fast procedure for the Golgi silver impregnation

- 1 Fix one to two days in 10 per cent formalin
 - 2 Transfer to Muller's solution or 2.5 per cent aqueous potassium bichromate for two days at 34° C
 - 3 Rinse in 2 per cent AgNO₃ and transfer to same for one or two days at 34° C
- Embed and mount as usual

Although one is not certain which elements will show up better this is a quick, perfectly dependable method for class preparations. Landau's sympathetic cells of the cerebellum generally hard to treat are frequently impregnated as well as other ganglionic cells

TISSUE A Modification of the Golgi Method Aoyama, F Ztschr wissenschaft Mikr 46 489 1930

The cadmium formol method presents clearly the Golgi apparatus in various cells and succeeds in relatively large pieces

- 1 Fix small pieces of tissue in the following fluid three to four hours

Cadmium chloride	1
Formol neutral	15
Distilled water	85

- 2 Rinse quickly in two changes of distilled water and transfer to 1.5 per cent solution of silver nitrate for ten to fifteen hours at 22° C

- 3 Rinse quickly in two changes of distilled water preferably in a dark room and transfer for five to ten hours to the following reducing solution

Hydrochinon	1
Formol, neutral	15
Distilled water	85
Sodium sulphite	0.1 to 0.15 (a quantity which will produce a yellow tinge)

- 4 Wash thoroughly in tap water run up through the alcohols, embed and cut 3 to 4 micra thick

- 5 Mount and stain as desired with carmin or hematoxylin-eosin

The length of silver impregnation depends upon the temperature and may be regulated accordingly. Epithelial cells of the alimentary tract or of the urogenital system require only one to two hours fixation. Silver nitrate in 0.5 per cent solution also gives satisfactory results if allowed to act for thirty six to forty eight hours. Various organs of amphibians, reptiles, birds, and mammals may thus be prepared. Cold blooded animals seem to require longer fixation and impregnation than do warm blooded ones.

LACTIC ACID Colorimetric Determination of Klein F and Melka, J Wien med Wchnschr 7 53, 1929

Place 10 c.c. of 1 per cent phenol and 1 c.c. of 3 per cent ferric chloride solution in each of 2 comparison tubes

To one tube add 0.5 per cent lactic acid. To the other add drop by drop filtered gastric juice until a match is obtained

MUSEUM SPECIMENS Preservation in Color, Rohdenburg, G L Arch Path 94 874,
1930

The appended formula is for approximately 1 liter of solution

Potassium sulphate	0.5 gm
Potassium nitrate	2.25 gm
Sodium chloride	4.5 gm
Sodium bicarbonate	9.0 gm
Sodium sulphate	11.0 gm
Sodium acetate	7.5 gm
Chloral hydrate	25.0 gm
Solution of formaldehyde U S P	25.0 cc
Iso propylalcohol (technical)	50.0 cc
Water	1000.0 cc

A glass tube which is connected with the illuminating gas supply is passed to the bottom of the container, and the gas permitted to bubble through the mixture slowly for about one hour. If the glass tube is 0.5 cm in diameter, then one bubble every second is enough gas flow. The gas is then turned off and the container immediately closed with a tightly fitting stopper.

The specimen to be preserved is wiped free of blood clots (it is not washed in water) and placed, preferably hung suspended, in a jar sufficiently large so as not to cause pressure at any point. Approximately ten times the volume of the specimen is sufficient fixative. While the color is immediately fixed, fixation should not be for less than four days, the specimen may be left in the solution indefinitely. It is best, before placing the specimen in the fixing fluid, to prepare it as it is finally to appear, by sewing it to sheet celluloid, which is practically invisible when the specimen is mounted in gelatin.

If mounted in gelatin or a similar gel, then the used fixative is filtered and returned to the original stock. If the stock solution is not used up, gas should be permitted to bubble through it for about thirty minutes once a month, or should this be forgotten, then gas should be introduced for thirty minutes at least two hours before a specimen is placed therein. Specimens left in the fixing fluid do not become hard and stiff.

For mounting in gelatin, the method is as follows. One liter of water is brought to the boiling point, the heat is removed and 100 gm of gelatin of exceptional purity is rapidly added in small pieces. The mixture is stirred until complete solution has occurred and then from 3 to 4 teaspoonfuls of activated charcoal (the author has used Darco decolorizing carbon) is added for each 100 cc of the gelatin. The solution is to be stirred and kept hot for five minutes. It is then filtered through a Buchner funnel with suction, the mat for the filter being either washed asbestos or a piece of snugly fitting, closely woven cloth, such as linen. The filtrate is refiltered until clear. Coarse filter paper without suction may also be used, but with suction, filtration of one liter takes about five minutes. This gives a gelatin that is water clear and practically colorless. The excess fixative having been permitted to drain off and the specimen having been placed in the chosen jar, 4 cc of 40 per cent formaldehyde is added to each 100 cc of melted gelatin, and the jar filled with this mixture. The resultant gel is practically irreversible.

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EDITORIALS

The Challenge of Rheumatism

EVERY physician carries in his mind's eye the picture of some disease or abnormal condition which he is sorry to see cross his office threshold. If a poll were taken rheumatism would rank high among such conditions thus reflecting the attitude of the average physician to a most disabling disease. This attitude is brought about by the confusion and complexity of the conditions which we group under the term rheumatism and by the frequent unsatisfactory results of treatment. The patient in turn is the sufferer.

Few physicians, however, realize the economic importance of rheumatism. There are few accurate statistics available for the United States although every clinic sees many such patients. One large clinic in this country reports that in 1927 5000 patients, or one fourteenth of the total number of admissions, suffered from one of the rheumatic diseases. In Great Britain, where accurate statistics are available, Sir Walker Kinnear, Controller of health and pension insurance, found in 1927 that among 15 000,000 industrial

workers in the insured group, rheumatic diseases came third on the list of diseases for which physicians were consulted. In the same year \$100,000,000, representing 34,000,000 weeks of disability was paid in disability benefits. One-fourth, or \$25,000,000, of this sum representing nearly one-sixth of the total period of disability was paid for disability due to rheumatic diseases. Such figures emphasize the fact that rheumatism is one of the major economic as well as humanitarian problems of medicine.

There has been more organized interest in rheumatism abroad than in the United States. In England serious attention has been given to the subject as evidenced for instance by the recent Bath conference on rheumatic diseases. Sweden is building several hospitals solely for the care of joint disease. In this country a few clinics for the study and treatment of rheumatism have been developed but relatively little general interest has been aroused in the ranks of the medical profession. This is not surprising when one considers that no problem in medicine is more difficult to handle or taxes more the ingenuity of the clinician. No disease touches on more fields in medicine or requires greater teamwork in determining causes and treatment.

The rheumatic problem presents a distinct challenge to the medical profession of the world. The needs are many: more hospital space should be available for the treatment of patients, more clinics should be established for the care of ambulant patients, more physicians are needed to give the subject serious thought and study, more research workers should be interested in both clinical and laboratory angles of the disease. The American Committee for the Control of Rheumatism, in cooperation with the International Committee for the Study of Rheumatism, can assist greatly in meeting these needs.

In emphasizing the needs of the rheumatic problem one should not lose sight of the fact that much is already known concerning rheumatism and that the intelligent application of knowledge now available will give most sufferers from the disease new hope. The more serious students of the disease now have an optimistic attitude, which is well justified by the results of treatment, and which has a most valuable psychologic effect on the patient.

The challenge of rheumatism must be met!

—R L H

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VALUABLE SUGGESTIONS FOR CONTRIBUTORS TO THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE

"The four rules for the preparation of an article will then be (1) Have some thing to say, (2) Say it, (3) Stop as soon as you have said it, (4) Give the paper a proper title"

Let your phraseology express one meaning and one only Be clear

Manuscript—Manuscripts should be typewritten, with wide margins, and double spaced, on one side of paper 8½ by 11 inches in size The original copy should be sent to the "Journal" and the carbon copy retained by the author Number the leaves consecutively, beginning with the title page Put your name and address on the manuscript

Illustrations—Illustrations should be clear, preferably pen and ink drawings Of photographs send a good print rather than a negative Have lettering parallel to the bottom and top margins, and of sufficient size to be clear if cut is to be reduced Tracings should be in black and white, avoid colors Write your name on back of each picture, number them in one series (Fig 1, etc) to the end, and indicate in margin of the manuscript about where each is to be printed See that the text references and "figures" correspond Legends for illustrations should be written on a separate sheet

Bibliographic References—Give only references actually consulted If an article is known only through an abstract give reference to the abstract in addition to that of the source References are printed to be of help in further reading, therefore, they must be complete, concise, and correct Follow the style of the "Index Medicus" and "Index Catalog of the Library of the Surgeon General's Office" Be conservative in the use of abbreviations

Arrangement—As authors are quoted in the text give each a number in the order of citation, and number the bibliographic reference with the same number Arrange the references in a list at the end of the article in the order of the numbers (see below), or arrange items in alphabetical order according to last names of authors, and distinguish between articles by the same author by the use of the date after his name in the text

Footnotes—Where an author wishes to use footnotes at bottom of each page instead of the bibliography at end of article, the footnotes should be written in the text, but separated from it by horizontal lines above and below, or better, place them at bottom of each page Use figures to indicate those footnotes, and number consecutively (1, 2, 3, etc) throughout the article If in addition to the bibliography mentioned above it is desired to use footnotes on certain pages, these can be indicated by an asterisk (*)

Final Reading—Let some one other than the author read the manuscript with these directions in mind

Shipment—Send manuscript flat, postage paid, to the editor, Warren T Vaughan, Medical Arts Bldg, Richmond, Va

Proof Reading—Read carefully, with special attention to spelling of names and bibliographic data Make corrections in the margin only with lines drawn from the revision to the point of change in the text Answer queries in the proof by making correction or crossing out query Verify your references from the sources, not from your carbon copy

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